SIMPLE EXPERIMENTS IN BIOLOGY

BY THE SAME AUTHOR

EVOLUTION OF MAN AND HIS CULTURE
(*Gollancz*)

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(*Gollancz*)

NATURE STORIES FOR CHILDREN
(Our Circle, *Co-operative Press*)

AN EXPERIMENTAL HUMAN BIOLOGY
(*Heinemann*)

SIMPLE EXPERIMENTS IN BIOLOGY

COMPILED BY

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TO

THOSE MANY, MOSTLY UNKNOWN,
EXPERIMENTERS, WHO ARE THE
TRUE AUTHORS .OF THIS BOOK

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PREFACE

THE compiler believes that many teachers, and especially those working in schools, will welcome a compact collection of simple experiments in biology. No effort is here made to cater for specialists working with automatic recorders and electrical centrifuges in lavishly equipped laboratories. The aim is the humble and even pedestrian one of helping teachers without specialised training to carry out experiments needing no expensive apparatus for the benefit of pupils of no more than average ability.

In order to keep the book within a reasonable compass, descriptions have been cut to the minimum. This volume is not so much a technical manual as a biological bran-tub; the reader takes his pick, but should make continual reference to the books mentioned in the bibliography for more detailed working instructions. Laboratory directions for observational work are not included, for there are several excellent books of this nature already available. Similarly, details of dissection routine and cytological method are excluded, while theoretical explanation is limited to a short introduction to each topic. Experiment, not theory or observation, is here the key-note.

War-time considerations of paper and expense have led to the reluctant decision to issue the book unillustrated. The alternative was to leave publication until the presumably piping days of peace, when paper may be less scarce and labour less expensive. But it is precisely war-time conditions, with large numbers of teachers struggling gamely to replace absent biology specialists, which make the need for such a collection of experiments the more urgent. Care has been taken that instructions should be as explicit as possible, and it is hoped that readers will generally be able to follow them without real difficulty, despite the absence of illustration.

In a book of this nature it is impossible to make adequate acknowledgement of debt. Every teacher of biology gathers as the terms go by, a store of experiments whose immediate

authorship is forgotten and whose original invention goes back into the mists. Somebody must first have thought of them, but none knows who. Here in this book is an experiment hazily remembered from a conversazione, here one turned up in an old school note-book which has somehow escaped destruction. Overpage an experiment derives from lecture notes, and is followed by others performed in this or that university laboratory. Many an author will recognise in these pages experiments occurring in his text-book (and doubtless in many others too!). Indeed, very little in this collection is original. It is in the main an anthology of plagiarism. Every biologist under whom the compiler has studied, every laboratory assistant he has known, the authors of very nearly every biology book he has read, many dozens of his friends and acquaintances—all have unwittingly been writing this book for years.

Special thanks are, however, due to Professor L. P. W. Renouf, who criticised the typescript; to Mr. W. W. Allen, Mr. L. J. F. Brimble, Mrs. E. J. Hatfield and Miss P. M. Taylor, who were good enough to criticise the proofs; and to Mr. G. E. Briggs, Miss L. M. Crump, Dr. D. L. Gunn, Professor Lancelot Hogben, Dr. Julian Huxley, Professor R. C. McLean, and Miss R. F. Shove, who kindly criticised the proofs of certain sections. To my wife and to Miss Whitehead of my publishers, deep gratitude is due for their never failing patience and consideration. The generosity with which all this help was given, makes it doubly necessary to emphasise that for any errors, omissions or inadequacies, the compiler alone bears responsibility.

Finally—the Central Council for Health Education is in no way responsible for the material compiled or the views expressed in this book.

CYRIL BIBBY

INTRODUCTION

THERE has of recent years been a very marked increase in biological teaching in this country. In the post-primary schools it has taken the form both of Biology as a separate subject, and of the biological aspects of General Science. There has also been a tendency in the post-certificate stage for Botany and Zoology as separate subjects to give way to an integrated course of Biology. Not unnaturally, teachers have been well-nigh overwhelmed by a veritable spate of new text-books. These, as might be expected, have been of varying calibre. In most cases however, the text has been largely descriptive, and what practical work was recommended mainly observational. Since unfortunately school teaching tends only too often to follow the text-book (an especially undesirable juxtaposition of cart and horse), many pupils spend some years studying biology without ever performing more than a meagre handful of experiments.

What then is the place of experiment in biological study? It should be a very central one, and as prominent as considerations of laboratory equipment will allow. Many experiments of course, may well be demonstrated by the teacher, but where convenient the pupils themselves should perform them. It is often a very useful procedure for the students to work in pairs, each pair doing a different experiment on the topic under study, and rotating each lesson to fresh experiments until all have been completed. This plan lessens considerably the demand both on apparatus and on the teacher's time in preparation.

With younger pupils at any rate, experimental procedure should be of the simplest. Only one point should be emphasised at a time, no elaborate methods of measurement or record should be allowed to obscure the essential aim of the experiment, and apparatus should be as large and impressive as possible. The place for electrical and mechanical recording

and precise micro-analysis is the university or research laboratory, and only rarely the school.

The heuristic method of experiment has many good points, and certainly it is wise that pupils should at any rate occasionally carry out a genuine piece of experimental investigation. But most teachers find it impossible and many consider it undesirable to follow this method exclusively. Often pupils will be required to perform an experiment to "prove" an already known fact, often merely to make an observation which scarcely ranks as experiment at all.

It is well therefore, for pupils from the first to enter in their notebooks the relevant heading—"To investigate", "To confirm" or "To observe"—thus clarifying their minds as to what exactly they are accomplishing. Notes should also invariably be dated, and systematically recorded under such headings as "Material", "Method" (with reasons for the particular procedure adopted), "Results" (simply, and where possible numerically, recorded), and "Conclusions" (including almost always an estimate of their degree of reliability and a statement of possible sources of error). Large and clear diagrams, without any insignificant complications, should form an important part of the notes. Notebooks interleaved with blank pages are therefore desirable, unless a loose leaf system is adopted in the case of older students.

One valuable method of encouraging an appreciation of scientific method, is an insistence upon the performance of control experiments. It is not usually possible of course, under school conditions, to carry out *all* the controls necessary for rigid proof, but it is only rarely that *some* control cannot be devised. This need for control is especially urgent in the biological sciences, since the living organisms under study are so much more complex and variable than the lifeless material of the physicist and chemist. Herein lies the special difficulty of biological experiment, and herein its special joy.

TOPIC A.

BIOPHYSICAL PRINCIPLES

Certain physical principles are so basic to the understanding of biology that their study should form part of a biology course.

The Colloidal State.

Protoplasm is a crystallo-colloidal system. One difference between the crystalloid and the colloid states is that in the former, dissolved substances exist in the form of ions, molecules or hydrated molecules, whereas in the latter the dispersed substance is in the form of larger particles generally between 1 μ μ and 0.1 μ in diameter. At the surface of these particles, surface energy considerations become important. Crystalloids and colloids may be separated by dialysis (i.e. diffusion of the crystalloid through a membrane). Some organic chemicals have such large particles that even a "true" solution exhibits certain colloidial properties.

Colloidal systems sometimes exist as sols (liquids), sometimes as gels (pseudo-solids). The transition between the two states is often reversible. Sols may be coagulated by the action of various salts in reducing the electric repulsion between the charged particles, or by heat. Gels will often imbibe water, becoming hydrated and in some cases changing eventually into sols.

Diffusion.

In all living organisms there is continual diffusion of salts, sugars, amino-acids, gases, etc., from regions of higher concentration to regions of lower concentration. Diffusion takes place most quickly in gases, fairly quickly in liquids and colloidal gels, and slowly in solids.

Great care should be taken to avoid any confusion of the distinct phenomena of diffusion and osmosis. Particularly in the consideration of the osmotic entrance of water and the independent diffusive entrance of salts into root hairs, is this vigilance necessary.

Osmosis.

Certain membranes are semi-permeable (i.e. they allow the passage through them of solvents such as water, but not of dissolved substances such as sugars or salts). When two solutions are separated by a semi-permeable membrane, water tends to pass through it from the less concentrated to the more concentrated solution. The pressure which causes this movement (osmosis) is called the "osmotic pressure".

The boundary between the vacuole and the surrounding protoplasm of living plant cells acts as a semi-permeable membrane, while many animal membranes are also semi-permeable. The semi-permeability of living membranes may be destroyed by poisons, heat, etc. In plant cells, the inward pressure of the cell wall acts against the osmotic pressure, so the net suction pressure of the cell is equal to their difference. When plant cells are placed in a solution of high osmotic pressure, water leaves them, and the protoplasm shrinks from the cell wall (plasmolysis). The osmotic pressure of cell sap equals that of a solution which just plasmolyses the cell. Animal cells under like circumstances tend to shrink as a whole.

Rigidity.

All organisms above a certain quite small size (except some living in and supported by water) need rigidity. This may be achieved by means of a skeleton, either internal (as in fish, quadrupeds, birds and many plants) or external (as in insects, crustaceans, etc.). It may however be achieved by means of turgor (as in many plant structures and to a certain extent in animals). Turgor in plants is due to the mutual pressure of cells distended as a result of the osmotic absorption of water, and is responsible for most rigidity of smaller herbs.

Skeletons normally have a structure showing considerable economy in the achievement of mechanical strength (c.f. girders in steel-frame buildings). Internal skeletons of animals usually consist of collagen (a gelatin-like material) more or less impregnated with calcium phosphate etc. External animal skeletons consist largely of calcium carbonate (e.g.

snail), chitin (e.g. insects) or chitin impregnated with calcium salts (e.g. crayfish). Plant skeletons usually consist basically of lignified cellulose, often with salts of calcium etc.

Surface Properties.

Molecules at the surface of a liquid are usually arranged in such a way that the surface appears to be under tension, and acts as a film or membrane. This is important to pond insects (e.g. water boatman). Similarly surface tension exists at the interfaces of all immiscible liquids, solids and gases.

The surface tension is an expression of the surface energy, which is of fundamental importance in living things. Surface energy, in common with other forms of potential energy, tends towards a minimum. Thus liquid surfaces tend to take a spherical shape (the sphere being the geometrical form with minimum surface for a given volume), and when this tendency is in conflict with another (e.g. the gravitational tendency to reach the lowest level), a compromise shape results. Substances such as fats which reduce surface energy (and tension) tend to accumulate at interfaces.

Surface tension is the cause of capillarity—the tendency for liquids to rise in narrow tubes or spaces. Capillarity is important in the soil, where there are small spaces between the particles. The rôle of soil capillarity in "drawing up" water absorbed by plant roots, has however, been much exaggerated.

Experiments are classified as follows:—

A1. The Colloidal State.
A2. Diffusion.
A3. Osmosis.
A4. Turgidity.
A5. Skeletal Rigidity.
A6. Surface Properties.

See also experiments F 1a, b; J 3h.

A 1. The Colloidal State.

N.B.—In all experiments on the colloidal state, glassware must be scrupulously clean.

A 1a. Into two test tubes place (a) granulated sugar, (b) powdered glue, to a depth of ¼″. Add water, and heat to dissolve. In (a) the solution is clear (crystalloid solution), but in (b) turbid (colloid solution). Allow to cool. In (a) there is no change, in (b) a gel is formed.

A 1b. Pour 10 ml. acetone chlorophyll extract (B 5a) into 100 ml. distilled water, stirring continually. The colour becomes purer green, and the fluorescence disappears as a colloidal solution of chlorophyll is formed.

A 1c. Make a fairly concentrated solution of ferric chloride, and filter. Pour 5 ml., while stirring, into 500 ml. boiling distilled water. A rich red brown sol of ferric hydroxide is formed.

A 1d. Add 1 ml. 1% gold chloride ($AuCl_3.2H_2O$) solution to 100 ml. distilled water. Now add 1 ml. 0·5% tannic acid solution and mix. On standing, a deep red colloidal suspension of metallic gold is obtained.

A 1e. Observe microscopically some dilute indian ink. The particles may be seen executing the "Brownian movement".

A 1f. Dissolve 4 gm. starch and 0·5 gm. sodium chloride in 100 ml. water, making a sol. Pour into a parchment thimble hanging in distilled water, previously tested by silver nitrate solution for absence of chloride. After an hour, test the water again for chloride, and by means of iodine solution for starch. The former test is positive, the latter negative, since the crystalloid passes through the membrane but the colloid does not.

A 1g. Dissolve white of egg in twice its volume of water. Now add to separate parts of the solution, concentrated solutions of lead acetate and copper sulphate. Protein is precipitated from the colloidal condition.

A 1h. Place 2-3 ml. colloidal gold solution in each of a series of test tubes. Add solutions of N/10 sodium chloride, calcium chloride, etc. drop by drop, recording the number of drops required to change the colour to blue (i.e. on the way to precipitation). The higher a metal's valency in a salt, the greater its effect.

A 1j. Pour a little white of egg into a test tube and heat. The sol becomes white and opaque, owing to coagulation.

A 1k. Measure by displacement the volume of ten dried peas. Allow to soak for a day and remeasure. It is now greater, owing to imbibition of water by the gel of the seed.

A 1l. Place a small measured rectangle of dry sheet-gelatine on a considerably larger piece of glass. Flood the gelatine with water, and leave for a day. Remeasure. A considerable expansion of the gelatine will have taken place, owing to imbibition of water. Upon drying, the gelatine reshrinks.

A 1m. Repeat experiment A 1l with a portion of dried thallus of carragheen (the sea-weed *Chondrus crispus*).

A 1n. Cut a transverse section of a stipe of dry *Laminaria* and mount in about 90% alcohol. While observing microscopically, irrigate with water by placing a drop at one side of the cover glass and a piece of blotting paper at the other. As imbibition occurs, an enormous thickening of the cell walls is visible.

See also experiments A 5f; C 3c, d; E 2d-g; F 3k.

A 2. Diffusion.

A 2a. Place 2-3 ml. bromine in a porcelain crucible, and cover with an inverted gas jar. Despite its density being greater than that of air, the bromine vapour slowly spreads throughout the gas jar by diffusion. A white paper background makes the experiment ea.ier to observe.

A 2b. Invert an unglazed porcelain jar and pass a glass tube through a rubber bung in its neck. Connect the glass tube to a manometer containing a coloured liquid. Invert a beaker of hydrogen over the porcelain jar. The manometer indicates an increase of pressure in the jar, owing to hydrogen diffusing into it more rapidly than air diffuses out.

A 2c. Introduce a crystal of potassium permanganate carefully to the bottom of water in a tall narrow cylinder. The crystal dissolves and the permanganate gradually diffuses throughout the liquid.

N.B.—avoid disturbing convection currents.

A 2d. Prepare a warm gelatine-in-water sol, and add a few drops of colourless phenolphthalein indicator. Pour into a warm test tube and allow to cool. When the sol has set to a gel, invert the tube with its end dipping into 10% sodium hydroxide solution, and clamp in position. As the alkali diffuses, the gel becomes pink owing to the reaction of the alkali with phenolphthalein.

A 2e. Remove the lung of a dead frog, and tie up with thread. Weight with a small piece of lead, and drop into a beaker of "soda water". The lung distends with carbon dioxide, which diffuses through into it.

A 2f. Repeat experiment A 2e with a bag made from the skin of a dead frog's leg.

A 2g. Repeat experiments A 2e and A 2f, using a beaker of hydrogen peroxide solution. The bags become distended with oxygen, which is liberated from the peroxide and then diffuses in.

A 3. Osmosis.

A 3a. Fold a cellophane jam cover like a filter paper, and support on a filter ring, with the lower part of the cone dipping into water. Into the cone pour concentrated sugar solution (e.g. golden syrup), and mark its level with a pencil. Leave

for a few hours, when the liquid level will have risen in the cone by the process of osmosis.

A 3b. A semi-permeable collodion membrane for demonstrating osmosis may be prepared by dipping the end of a thistle funnel into B.D.H. collodion preparation, withdrawing, and holding in the air to dry. The funnel is then filled with a solution, and held by a retort with the membrane dipping into water.

A 3c. Drop a small crystal of copper chloride into a beaker of 2% potassium ferrocyanide solution. A membrane of copper ferrocyanide is formed around the crystal, and as osmosis proceeds the membrane swells and periodically bursts.

A 3d. Peel a large potato, and slice its ends flat. Scoop out its interior so that a "cup" is made, with a wall about $\frac{1}{2}$" in thickness. Add some granulated sugar and stand in a trough of water. After a day, liquid will have risen in the potato cup, by the process of osmosis. Set up in the same trough as controls: (1) an empty potato cup, (2) a parboiled (i.e. killed) potato cup containing sugar.

A 3e. Remove shells from two eggs, by leaving a day or two in dilute hydrochloric acid (about 10% by volume). Wash well in water and measure their volumes by displacement. Place one egg in distilled water, one in concentrated sodium chloride solution, and leave for two days. Remeasure their volumes. The first egg is enlarged and the second diminished, because the lesser or greater solute-concentrations of the surrounding liquids compared with the egg contents, lead to inward or outward osmosis. Instead of eggs, grapes may be used, care being taken not to damage the "stalk" end.

A 3f. Cut thin sections from a fresh beetroot, and examine microscopically, mounting in water. Immerse other sections in 10% sucrose solution for 10 minutes, and examine microscopically, mounting in the same solution. Many cells will be plasmolysed. Instead of beetroot, *Spirogyra* filaments or epidermal strips from *Pelargonium* petals may be used.

A 3g. Repeat experiment A 3f, using 5% glycerine solution. Plasmolysis occurs, but the cells regain their turgor owing to the gradual entry of glycerine into the cell vacuoles.

A 3h. Tie a semi-permeable animal membrane (e.g. pig's bladder) over one end of a glass tube about 3″ long and 1″ in diameter. Nearly fill the tube with a concentrated sugar solution (e.g. golden syrup) and fill up with an easily visible liquid (e.g. black ink). Insert a bung carrying a long narrow glass tube, and note the level of the liquid in the tube. Dip the membrane into a trough of water (containing phenol to inhibit bacteria) and note the rise in level of the liquid in the tube, as water passes by osmosis through the membrane into the syrup.

A 3j. Make up in beakers M, M/2, M/4, M/8, M/16 solutions of sucrose. With a cork borer, make from a large potato 5 cylinders, each about 10 gm. in weight. Dry carefully with blotting paper (do not apply pressure) and weigh. Place one cylinder in each solution, and leave for 2 days. Then dry again and re-weigh. If the weight is increased owing to the absorption of water, then the suction pressure of the potato is greater than the osmotic pressure of the solution, and vice versa. Whence calculate the suction pressure of the potato. By cutting the cylinders into thin slices, the experiment may be considerably speeded up to give results in 1–2 hours.

A 3k. Split a scape of dandelion or daffodil into 6 pieces, and make a drawing of each, indicating the degree of curvature. Place the pieces in sugar solutions of various concentrations ranging from 2–16%. After they have ceased to alter in curvature (owing to the cessation of inward or outward osmosis), redraw. Superimpose the two sets of drawings, and observe which solution causes neither curling nor uncurling. This has an osmotic pressure equal to the suction pressure of the dandelion or daffodil cells.

A 3l. Place *Spirogyra* or other plant cells into sugar solutions of various concentrations ranging from 2–16%. After a few minutes observe microscopically. The solution in which

plasmolysis only just occurs (or occurs in 50% of the cells) has an osmotic pressure equal to the osmotic pressure of the cell sap.

A 3m. Place drops of blood in distilled water and in 5% salt solution and examine microscopically. The red corpuscles of the former drop swell, and those of the latter shrink, owing to osmotic movement of water into or out of the cells.

A 3n. In a series of 5 test tubes place solutions of sodium chloride of various concentrations (e.g. 0·1%, 0·3%, 0·5%, 0·7%, 0·9%). To each add 3–4 drops of defibrinated blood (H 3e), and stand for an hour. Compare the rates of haemolysis (as indicated by increasing depths of colour) in the solutions. "Physiological" salt solution is 0·9%, and this concentration causes no haemolysis.

A 3p. Cut a thin slice of beetroot, and wash away any liberated cell sap. Suspend with a thermometer in a beaker of water, and heat very slowly. Note the temperature at which the protoplasmic membrane, being killed, allows the passage of red sap into the water. Instead of being heated, the membrane may be rendered permeable by the addition of chloroform.

See also experiments A 4a, b, f; F 5k; J 2b.

A 4. Turgidity.

A 4a. Cut several turgid herbaceous plants. Place some with their cut ends in fresh water, and others in concentrated salt solution. Leave for an hour. The first set of plants remain turgid, the second become flaccid owing to osmotic loss of water.

A 4b. With a cork borer, make several cylinders of potato. Cut to 1″ in length and measure their mean diameter. Place some in distilled water, and some in concentrated sodium chloride solution. Examine after a day. The former cylinders become larger and turgid, the latter smaller and flaccid, owing to osmosis of water into, or out of, the cells.

A 4c. Cut a long straight stick of rhubarb, and measure its length. Strip off the whole of the outer coat. Remeasure its length. The length is greater, owing to removal of restraint by the outer layer.

A 4d. From the freshly exposed end surface of a thick stick of rhubarb, cut out carefully a cylindrical disc. Leave for a few moments, and attempt to replace. This is difficult, owing to expansion of the disc, once the restraint exercised by the outer layer has been removed.

A 4e. Split a dandelion scape longitudinally into four. The quadrants curl up, with the epidermis on the concave side. This demonstrates that the epidermis, under tension, normally restrains the expansive tendency of the inner cells.

A 4f. Place two of the strips into water, and two into concentrated salt solution. The former curl still more, while the latter uncurl, owing respectively to osmotic gain and loss of water by the inner cells.

A 4g. Split the apical centimetre of a 3–4 cm. root of broad bean (*Vicia faba*) by means of a razor blade, and place in lukewarm water. The halves bend slightly inwards after a while, since the tissue tending to expand is in this case external.

A 5. Skeletal Rigidity.

A 5a. Remove as much flesh as possible from a frog's skeleton, and then leave it soaking in water until most of the remaining flesh has left the bones. Bleach by exposing to the sun for some days, thus obtaining a clean white skeleton.

A 5b. Break a large long mammalian bone, and observe the space containing marrow. The stresses are borne by the hollow cylinder of bone, as in a hollow steel mast.

A 5c. Place a bone on a tripod and heat very strongly for 30 mins. in a bunsen flame. After heating, the bone is more

brittle, owing to combustion of the collagen. Now place the ignited bone in concentrated hydrochloric acid. It effervesces and dissolves away as the calcium salt reacts with the acid.

A 5d. Place a bone in hydrochloric acid for a couple of days. Then remove and wash well in water. The bone is more pliable, owing to the loss of the calcium phosphate. Now dry, place the soaked bone in a crucible and ignite. It burns away as the collagen undergoes combustion.

A 5e. Repeat experiments A 5c and A 5d quantitatively, weighing the bone before and after initial treatment. Bone is usually about 67% by weight salts, and 33% organic material.

A 5f. Boil some bones for 4–5 hours in just enough water to cover them. Decant the liquid and allow to cool. It sets to a jelly owing to the gelatine formed from the collagen of the bones.

A 5g. Crush a bone, dissolve as far as possible in dilute nitric acid, and decant. Test the solution for calcium and for phosphate as in experiments B 4c, e.

A 5h. Boil 20 gm. sodium carbonate and 10 gm. calcium hydroxide with 100 ml. water until as much as possible has dissolved. Cool, filter and place some leaves in the filtrate. Boil for an hour, wash well in water, and rub the leaves gently between finger and thumb. Dry the remaining leaf skeleton and mount between glasses.

A 5j. Place a piece of wood (e.g. matchstick) in a solution of aniline sulphate. The wood turns bright yellow owing to the presence of lignin.

A 5k. Repeat experiment A 5j using a mixture of phloroglucin solution and concentrated hydrochloric acid. The wood turns bright red owing to the presence of lignin.

A 5l. Cut a transverse section of the stem of a plant (e.g. white deadnettle, *Lamium album*) and stain the lignin as in

experiment A 5j or A 5k. Observe the efficient girder-like disposition of the lignified skeletal tissue.

A 5m. Boil a celery petiole and tease out in a solution of aniline sulphate or phloroglucin and hydrochloric acid. The stained lignified elements may then be observed microscopically.

A 6. Surface Properties.

A 6a. Float a piece of tissue paper on water and place gently on it a clean steel needle. The paper will sink, but the needle (relative density 7·8) will float on the surface.

A 6b. Make a soap film on a wire ring across which is tied a piece of loose thread. Puncture the film on one side of the thread by means of a hot needle. The film contracts to a minimum area, stretching the thread.

A 6c. Spill a little mercury into a flat dish from a height. Note that the smaller globules (of little weight) are almost spherical, while the larger ones (in which the effect of gravity is considerable compared with surface forces) are bun-shaped.

A 6d. Prepare an alcohol-water solution of the same density as olive oil. Run olive oil gently into it, by a pipette dipping below the surface. The oil forms a large yellow sphere, being relieved by the solution of the effect of gravity.

A 6e. Take a series of capillary tubes of various bores and cleanse thoroughly in dilute caustic soda and then water. Stand in a dish of water. The water rises higher in the narrower tubes.

A 6f. Repeat experiment A 6e, using mercury instead of water. In this case the meniscus is convex and the mercury falls lower in the tubes than in the dish.

A 6g. Place a stick of blackboard "chalk" in ink, and note the capillary rise of ink.

A 6h. Pour some paraffin oil on the surface of the water in an aquarium containing gnat larvae etc. The surface tension is reduced and the animals sink and cannot obtain air.

A 6j. Place in boiling tubes about 10 ml. of 0·01% solutions of eosin, methylene blue, etc. To each add 1 gm. powdered charcoal, and shake. The dyes are adsorbed, and the solutions become colourless.

A 6k. Swill out a flask with methylene violet and then rinse with distilled water until the water comes away colorless. Now rinse with methylated spirit. This comes away coloured, since it removes stain adsorbed onto the glass and not removed by water.

See also experiments B 5b, c; C 2e–g.

TOPIC B.

BIOCHEMICAL TESTS

Little point is served by carrying out innumerable biochemical tests for the same type of substance. The aim should be to carry out relatively few tests, but to *understand* them. A selection should therefore be made from the tests given below.

Fats and oils are esters of alcohols with fatty acids, water being eliminated during their formation. The alcohol concerned is usually glycerol and the acid either palmitic, stearic or oleic. Palmitic acid has the formula $C_{15}H_{31}COOH$, and is a waxy white solid. Stearic acid is rather similar, and has the formula $C_{17}H_{35}COOH$. Oleic acid is an oily liquid, with the formula $C_{17}H_{33}COOH$.

Glycerol has the formula $C_3H_5(OH)_3$, and each of its three "OH" groups may combine with the "COOH" group of a fatty acid, water (H_2O) being liberated. The three "COOH" groups may be from three molecules of the same acid, or from different acids.

Glycerol palmitate is also known as tri-palmitin, glycerol stearate as tri-stearin, and glycerol oleate as tri-olein. Naturally occurring fats may contain a proportion of all three, and also of "mixed" glycerides. Tri-olein has a comparatively low melting point, and occurs abundantly in olive oil, to a certain extent in lard, and only slightly in suet.

The formation of a fat can be illustrated by the following equation:—

palmitic acid +	glycerol	$\longrightarrow$ glycerol palmitate +	water
$3C_{15}H_{31}COOH$	$C_3H_5(OH)_3$	$(C_{15}H_{31}COO)_3C_3H_5$	$3H_2O$

Under suitable conditions the above reaction may occur in the reverse direction. The fatty acid thus produced may combine with an alkali such as sodium or potassium hydroxide, producing a sodium or potassium salt (i.e. a soap).

palmitic acid +	sodium hydroxide	$\longrightarrow$ sodium palmitate +	water
$C_{15}H_{31}COOH$	$NaOH$	$C_{15}H_{31}COONa$	H_2O

While the term "fat" is used to include oils of similar chemical constitution (e.g. olive oil), it does not include substances of somewhat similar physical properties but quite different chemistry (e.g. vaseline).

Other groups often confused with fats are the "essential oils", the waxes and the sterols, but these will not normally be studied in an elementary course.

Carbohydrates, like fats, consist only of carbon, hydrogen and oxygen, but the elements are combined in a different way, the hydrogen and oxygen being in the proportion of two atoms to one. (Not all substances with such proportions, however, are carbohydrates. Note for example, lactic acid $C_3H_6O_3$). Monosaccharides, or simple sugars, may contain from three to seven carbon atoms, being correspondingly called trioses, tetroses, pentoses, hexoses and heptoses.

Hexose sugars contain six atoms of carbon and have the formula $C_6H_{12}O_6$. These atoms may be variously arranged, forming several different sugars such as glucose (grape sugar or dextrose), fructose (fruit sugar or laevulose) and galactose.

When two monosaccharides combine together, with the elimination of water, disaccharides are formed, as in the following equation:—

$$\underset{C_6H_{12}O_6}{\text{monosaccharide}} + \underset{C_6H_{12}O_6}{\text{monosaccharide}} \longrightarrow \underset{C_{12}H_{22}O_{11}}{\text{disaccharide}} + \underset{H_2O}{\text{water}}$$

The particular disaccharide formed depends upon the two monosaccharides combining. Thus:—

$$\text{glucose} + \text{fructose} \longrightarrow \underset{\text{(cane sugar)}}{\text{sucrose}} + \text{water}$$

$$\text{glucose} + \text{glucose} \longrightarrow \underset{\text{(malt sugar)}}{\text{maltose}} + \text{water}$$

$$\text{glucose} + \text{galactose} \longrightarrow \underset{\text{(milk sugar)}}{\text{lactose}} + \text{water}$$

This process, under suitable conditions such as occur during digestion, may be reversed, the disaccharide being hydrolysed by water to produce one or more monosaccharides (hexoses).

Many sugars (e.g. glucose, fructose, maltose, lactose) act as reducing agents. Their reduction of copper salts to cuprous oxide is the basis of several tests e.g. Fehling's, Trommer's, Benedict's.

By a continuation of the process of elimination of water, large numbers of monosaccharides may eventually be combined together in large molecules called polysaccharides, of which starch, inulin, glycogen and cellulose are the best known examples. The formulae of starch, glycogen and other hexosans are usually given as $(C_6H_{10}O_5)_n$ and those of pentosans as $(C_5H_8O_4)_n$, where n has very high values. By hydrolysis, polysaccharides may be split down into di- and then monosaccharides.

Starch consists of at least two, and probably more components. Amylo-amylose gives a clear non-viscous colloidal solution in cold water, and a pure blue colour with iodine. Amylo-pectin, which apparently always contains phosphoric acid in combination, is insoluble in cold water but in hot water swells up. It is responsible for the opalescence and viscosity of starch solution, and gives a violet coloration with iodine. Starch grains normally contain amylo-amylose, covered by a layer of amylo-pectin.

When starch is hydrolysed by enzymes, most of it is converted to maltose. This probably takes place in two ways, *via* a series of non-reducing polysaccharides called amyloses, and another reducing series called dextrins. When the hydrolysing agent is an acid, the maltose is further split into glucose.

Proteins, unlike fats and carbohydrates, contain other elements as well as carbon, hydrogen and oxygen. Nitrogen is always present, often together with sulphur and phosphorus and sometimes metals such as iron. Just as polysaccharides are built up from large numbers of monosaccharides, proteins are generally believed to be made up of large numbers of simple nitrogen-containing substances called "amino-acids"; and just as in the carbohydrates there are intermediate saccharides, so there are peptides intermediate between amino-

acids and proteins. Peptides are similarly an intermediate stage in the hydrolysis of proteins to amino-acids.

$$\text{amino-acids} \rightleftarrows \text{peptides} \rightleftarrows \text{proteins}$$

One simple aliphatic amino-acid is glycine (CH_2NH_2COOH), another alanine (CH_3CHNH_2COOH). There are also aromatic amino-acids such as tyrosine ($C_6H_4OHCH_2CHNH_2COOH$) and tryptophane ($C_8H_5NHCH_2CHNH_2COOH$). Amino-acids are linked together by the "NH_2" group of one combining with the "COOH" group of another, with the elimination of a molecule of water, giving the peptide linkage —CO—NH—

e.g.

$$\underset{CH_3CHNH_2COOH}{\text{alanine}} + \underset{CH_2NH_2COOH}{\text{glycine}}$$

$$\longrightarrow \underset{CH_3CHNH_2CONHCH_2COOH}{\text{alanylglycine}} + \underset{H_2O}{\text{water}}$$

The resulting dipeptide is clearly still an amino-acid, and therefore capable of further linkage.

The classification of proteins is still the subject of dispute, but the following are some of the better known groups:—

Protamins—basic proteins rich in nitrogen and yielding only a few amino-acids. They occur in ova and spermatozoa.

Albumins—soluble in water and dilute salt solution, and coagulated by boiling.

Globulins—insoluble in water, but soluble in dilute salt solution, and coagulated by boiling.

Glutelins—insoluble in neutral solutions, but soluble in dilute acids and alkalies. They occur in vegetables.

Scleroproteins—insoluble in most reagents. They occur in skeletal tissues of animals (e.g. keratin, collagen).

Phosphoproteins—rich in phosphorus (e.g. casein of milk, vitellin of egg yolk).

Conjugated proteins—compounds of protein and non-protein groups—e.g. haemoglobin (protein+pigment), mucin (protein+carbohydrate), nucleoproteins (protein+nucleic acid).

Proteins tend generally to have high molecular weights and to show colloidal properties. They are amphoteric, tending to combine both with free acids and free bases, thus exerting a buffer effect in the tissues.

Mineral Salts are present in all living organisms, both animal and plant. Apart from their effects on living processes by causing osmosis and differences of permeability, and affecting the hydrogen ion concentration of the tissue fluids, many salts have quite specific effects.

Certain salts provide essential components of chemical compounds necessary for life. Thus nitrates, phosphates and sulphates provide the plant with nitrogen, phosphorus and sulphur which may be built up into proteins; calcium is built into the skeleton of many animals, and as calcium pectate into the cell walls of many plants; phosphorus into bones and the various phosphorus compounds which are concerned in the oxidation processes of the cell; magnesium into chlorophyll, iron into haemoglobin and copper into haemocyanin; while iodine is an element of the hormone thyroxin, and chlorine of the stomach acid.

Other salt elements, while not themselves forming part of the final products, are necessary for the continuing of certain processes. Thus iron is necessary for the production of chlorophyll, and copper for that of haemoglobin. Magnesium is necessary for the fermentation of yeast and the respiratory breakdown of glycogen, and also for the proper formation of bone and the preservation of good nervous tone. Calcium is necessary for nervous health and for the clotting of blood, and along with sodium for heart-contraction, while heart-relaxation depends upon the presence of potassium. It has been discovered that certain mineral elements must be present in traces to prevent the onset of some diseases, in both animals and plants. For example, copper prevents "Gingin" disease of sheep, and cobalt "bush disease" of the same animals; while boron, manganese, copper and zinc have been proved essential to the normal growth of many crops. It is, therefore, a matter

of importance to be able to detect mineral salts in small quantities.

Pigments occur in most living things, both animal and plant. The most important plant colouring matter is chlorophyll—that concerned in photosynthesis. Chlorophyll is actually a mixture of four pigments:—

chlorophyll a:—(blue-green)—$C_{55}H_{72}O_5N_4$ Mg.
chlorophyll b:—(yellow-green)—$C_{55}H_{70}O_6N_4$ Mg.
xanthophyll:—(yellow)—$C_{40}H_{56}O_2$.
carotin:—(yellow-orange)—$C_{40}H_{56}$.

Xanthophyll also occurs in egg yolk and yellow feathers, while carotin is present in many flowers, fruits and roots.

Green algae contain only chlorophyll, but blue-green algae contain also phycocyanin (blue), red algae phycoerythrin (red), and brown algae phycophaein or fucoxanthin (brown).

Most red, purple and blue plant structures owe their coloration to anthocyanin pigments which are dissolved in the cell sap. The actual coloration produced depends upon factors such as hydrogen iron concentration and sugar and salt content of the sap. Thus flowers may differ in colour according to the type of soil, the temperature, and other physico-chemical factors.

Many yellow and white flowers owe their coloration to anthoxanthin (flavone and flavonol) sap pigments. The yellow and white pigments are very closely related. Thus flowers may be white or yellow—depending upon quite simple changes in the chemical processes of the cells.

Brown colours may be due to flavones, xanthophyll etc. but are often caused by tannins in the cell walls. Many tree barks, galls, etc. are very rich in tannin.

Black coloration of flowers is often due to melanin, a pigment occurring also in the skins of many animals.

Experiments are classified as follows:—

B1. Fats.
B2. Carbohydrates.
B3. Proteins.
B4. Mineral Salts.
B5. Plant Pigments.
B6. Common Foods.

B 1. Fats.

B 1a. Heat a small portion of lard in a porcelain crucible. Note the melting, followed by burning and evolution of acrid fumes—a simple test for fats.

B 1b. A very simple test for fats is that they leave a translucent mark when rubbed or heated on paper. This can be demonstrated with ordinary fats, and seeds such as those of the flax (linseed), the sunflower and the castor-oil plant.

B 1c. Shake up a little olive oil with water, and add a little Sudan III solution. Shake again, and then allow to settle. The pigment is taken up selectively by the oil, suggesting a test for fats. Scharlach R is an alternative stain.

B 1d. Crush some linseed in a mortar with a pestle. Place in a test tube one-third filled with water, and boil. An oily layer collects on the surface. Add Sudan III, shake, and allow to settle. The oil is stained light red, confirming the suggestion made in experiment B 1c. Sunflower seeds are also suitable.

B 1e. Add a few drops of osmic acid solution to a little lard, sugar, egg albumen and vaseline. The lard takes on a brown-black appearance owing to the production of a fine precipitate of osmium by reduction of the acid (more correctly, osmium oxide). This is a test for fats.

See also experiments B 6a, h–l.

B 2. Carbohydrates.

B 2a. Fit up a hard glass test tube with a cork and a delivery

tube dipping into lime water. Place dry powdered carbohydrate in the tube, fix the tube horizontally, and heat strongly. The lime water turns milky, indicating production of carbon dioxide. Liquid drops collect in the test tube, and these can be shown to be water by means of white anhydrous copper sulphate powder, which turns blue.

N.B.—This is not a *specific* test for carbohydrates.

B 2b. Mix a few drops of 1% alcoholic α-naphthol solution with a little carbohydrate solution. Run about 5 ml. concentrated sulphuric acid down the side of the test tube (care!). A violet coloration is produced at the junction of the two liquids, indicating a test for carbohydrate. Repeat with olive oil, salt solution, etc. to establish the specificity of the test. This test (Molisch's) is also given by many proteins containing a carbohydrate grouping.

B 2c. Add a few drops of iodine solution to test tubes containing solutions of starch, sugar, salt, egg albumen. The starch solution goes blue-black, indicating a specific test for starch. Heat the solution. The colour disappears, partially reappearing on cooling.

B 2d. Keep a 5% starch paste at −10° C. for 12–24 hours. The amylo-pectin separates out as a cotton-wool-like substance, the amylo-amylose being left in solution.

B 2e. Apply the iodine test to a little cotton wool. It does not turn blue. Now to the cotton wool add a little concentrated sulphuric acid. It now turns blue—a test for cellulose. Instead of iodine and acid, Schultz's solution may be used.

B 2f. Heat a lump of sugar in a porcelain crucible. Note the odour of caramel, followed by charring and eventually by complete combustion—a simple test for sugars.

B 2g. Add to glucose solution in a test tube half its volume of 20% caustic soda solution, and warm. A yellow-brown coloration occurs. Add 25% sulphuric acid until acid, when

a caramel odour is produced. (Moore's test.) Repeat with solutions of fructose, maltose, lactose, sucrose, starch, salt, etc. to establish the specificity of the test for reducing sugars.

B 2h. Add 2–3 ml. concentrated potash solution to a few drops of copper sulphate solution in a test tube. A precipitate is formed. Now add a solution of glucose. The precipitate re-dissolves, giving a blue solution. Boil, when a yellow or red precipitate is formed. (Trommer's test.) Repeat with other solutions as in experiment B 2g.

B 2j. Add a little Fehling's solution to a test tube containing a solution of glucose. The solution produces a red precipitate on boiling. Repeat with other solutions as in experiment B 2g.

B 2k. Repeat Fehling's test with sucrose solution, but first boil for a couple of minutes with a drop of dilute hydrochloric acid. The test is now positive, owing to the hydrolysis of the sucrose to hexoses (glucose and fructose).

B 2l. To 5 ml. of Benedict's solution in a test tube add about 10 drops of a very dilute solution of glucose. Boil vigorously for 2 minutes, and allow to cool. A precipitate is formed (red, yellow, or green depending on the concentration of sugar). Repeat with other solutions as in experiment B 2g.

B 2m. To 5 ml. glucose solution add a pinch of anhydrous sodium carbonate and 3–4 drops 5% copper sulphate solution and boil. A yellowish precipitate is formed (Cole's test). Repeat with other solutions as in experiment B 2g.

B 2n. To 2 ml. water add 1 drop 1% methylene blue solution, 2–3 drops 5% sodium hydroxide solution, and 2–3 drops glucose solution. Boil, when the colour disappears owing to the reduction of methylene blue by the glucose. Repeat with other solutions in experiment B 2g.

B 2p. Glucose may be estimated colorimetrically by the picric-picrate test. Fill up several test tubes to equal depths with glucose solutions of a range of different concentrations.

Add to each the correct volume of picric-picrate reagent and warm. A reddish-brown coloration is produced (due to formation of picramic acid) of a density proportional to the concentration of glucose. Compare the colorations with a series of standard tubes of known glucose content. (*N.B.*—*See* R 1q.)

B 2q. Test a green leaf for starch as follows. Kill by placing for a short time in boiling water. Now place in methylated spirit in a beaker, and insert beaker in a larger container in which is hot water (no exposed flame!). Repeat with fresh hot alcohol until the leaf is decolorised. To remove brittleness, leave the leaf in running water for a short time. Now place in iodine solution, and leave for a couple of minutes. Wash away excess iodine solution in running water. The parts of the leaf containing starch will be blue-black.

B 2r. Test a green leaf for sugars as follows. Cut up into shreds and cover with water in a mortar. Crush well with a pestle. Filter off liquid. About one-third fill a test tube with it, add 3ml. Fehling's solution and boil for 1 minute. If a reducing sugar (e.g. glucose, fructose) is present, a brick red precipitate will be produced. If test is negative, repeat with a fresh sample of liquid, but before adding Fehling's solution, boil with a little dilute hydrochloric acid. A positive result indicates the presence of a non-reducing sugar (e.g. sucrose).

B 2s. Cut fairly thick sections of a leaf (translucent when mounted) and warm in Fehling's solution or place in iodine solution, to test for sugar or starch. In the former case, microscopic examination shows shiny dark particles. In the latter case, dark blue or black coloration appears. In each case compare as controls, an untreated section of the leaf.

See also experiments B 6b–d, f, g, j; H 3j; J 5 b–d.

B 3. Proteins.

B 3a. Heat a small portion of boiled egg white in a porcelain crucible. Note the charring and characteristic "burning flesh" odour—a simple test for proteins.

B 3b. Add 1 ml. concentrated nitric acid to 3 ml. egg albumen solution in a test tube, and heat. A yellow coloration or precipitate is given. Cool, and add concentrated ammonia solution, when the coloration becomes orange (Xanthoproteic test). Repeat with solutions of starch, sugar, oil, salt, etc., to establish specificity of test for proteins.

N.B.—Gelatine also gives this reaction, since it contains an active group, phenylalanine.

B 3c. Add a little Millon's reagent to egg albumen solution in a test tube, and heat. A brick-red coloration or precipitate is given (Millon's test). Repeat with other solutions as in experiment B 3b. An improved version is described in R 1n.

N.B.—The reaction is due to the presence of tyrosine in the protein. Phenol also gives this reaction, but pure proteins of the gelatine type do not.

B 3d. Add an equal volume of 5% sodium hydroxide solution and a drop of 1% copper sulphate solution to egg albumen solution in a test tube. A violet coloration is produced (Biuret test). Repeat with other solutions as in experiment B 3b.

N.B.—Peptones (and often egg albumen) give a rose tint.

B 3e. Add 5–6 drops of 0·2% ninhydrin solution to 2–3 ml. egg albumen solution in a test tube, and boil. A blue coloration is produced (Ninhydrin test). Repeat with other solutions as in experiment B 3b.

B 3f. Add 2–3 drops of 0·1% formaldehyde and 2–3 drops 10% mercuric sulphate-sulphuric acid solution (R 1n) to 2–3 ml. egg albumen solution in a test tube. Mix, add 2 ml. pure sulphuric acid, and shake gently. A deep violet or purple coloration is produced. (Tryptophane test). Repeat with other solutions as in experiment B 3b.

N.B.—Pure proteins of the gelatine type do not give this reaction.

B 3g. Mix a protein with soda lime in a hard glass test tube

and heat. Ammonia is evolved, indicating the presence of nitrogen in the protein. It may be detected by smell, by turning red litmus blue, and by forming blue-white fumes at the mouth of a shaken bottle of concentrated hydrochloric acid.

B 3h. Boil 2 ml. of egg-white with a little 40% sodium hydroxide solution for a couple of minutes. Add a few drops of lead acetate solution. A black precipitate of lead sulphide indicates the presence of sulphur in the protein.

B 3j. Mix dry casein in a crucible with twice its volume of fusion mixture, and heat at first carefully and finally very strongly. Allow to cool and extract with 50% nitric acid. Add ammonium molybdate solution and boil. A yellow precipitate indicates the presence of phosphorus in the protein.

B 3k. Place a very young cress seedling in a watch-glass, and cover with Millon's reagent. Warm *gently* for several minutes, adding drops of water if necessary to prevent desiccation. Mount, in water on a slide, and examine microscopically. A reddish coloration near the root tip indicates the accumulation of protein in that region.

B 3l. Carry out Millon's and the Xanthoproteic test for proteins on finger or toe nail clippings and hair cuttings. (These contain keratin.)

See also experiments A 1g, j; B 2b; B 6e–g, j, k; H 3g, h.

B 4. Mineral Salts.

B 4a. Chop thoroughly a small handful of freshly cut grass. Place in a weighed porcelain crucible and weigh. Heat in a steam oven, cooling and weighing at intervals until the weight is constant. Whence calculate the dry weight and water content of the grass. Now heat the crucible, at first carefully and then strongly; finally heating on an open flame, stirring gently

to expose the contents to the air. Cool and weigh at intervals, until the weight is constant. Whence calculate organic content and ash content of the grass. Repeat with other plant materials, and compare the results.

B 4b. With a loop of platinum wire, take up a little plant ash. Place in a bunsen flame, and examine through a sheet of cobalt glass (which cuts out the yellow coloration due to sodium). A pale violet flame indicates potassium.

B 4c. Repeat B 4b without using cobalt glass, after moistening the wire with concentrated hydrochloric acid. A brick-red flame indicates calcium and bright yellow indicates sodium.

B 4d. Dissolve a little plant ash in dilute nitric acid. Add a little potassium ferrocyanide solution. A blue coloration or precipitate indicates iron. Confirm with potassium thiocyanate solution, which turns blood-red.

B 4e. Dissolve a little plant ash in dilute nitric acid. Add some nitric acid and ammonium molybdate solution. A canary-yellow coloration or precipitate indicates phosphate.

B 4f. Dissolve a little plant ash in concentrated hydrochloric acid. Decant, dilute, and if necessary filter. Add barium chloride solution to the filtrate. A white turbidity (best seen against a dark background) indicates sulphate.

B 4g. Dissolve a little plant ash as far as possible in water. Add a little 0·5% solution of diphenylamine in concentrated sulphuric acid. A blue coloration indicates nitrate.

B 4h. Dissolve a little plant ash in dilute nitric acid. Add a little silver nitrate solution. A white turbidity or precipitate indicates chloride.

See also experiments B 6m; C 1f, g; C 3f–h; H 3j; L 3b; L 4e–g.

B 5. Plant Pigments.

B 5a. Remove nettle leaves from their stalks, and allow to dry whilst spread out on paper. When dry, powder well. If the leaves are wanted for storage, dry them further at 30–40°C. Place some leaf powder on a filter paper on a suction funnel, add some 80% acetone, and after a few minutes soaking draw the liquid through by means of a filter pump. Repeat with several other equal volumes of acetone. Thus obtain a rich acetone-chlorophyll extract, deep blue-green in colour with a red fluorescence.

B 5b. Place a little chlorophyll solution in a tall gas jar, and in it stand a long strip of blotting paper. The various chlorophyll pigments travel up at different rates, and two green and two yellow bands may be seen.

B 5c. Pack a long wide tube evenly with powdered calcium carbonate. Now filter a chlorophyll solution through the tower, when the component pigments are separated by selective adsorption.

B 5d. Divide a batch of red algae into two parts. Boil one part in water for 10 minutes and leave in 95% alcohol for 1–2 weeks, in the dark. A green solution of chlorophyll is produced. Wash the second part in warm water for 1 hour and leave in a warm place for 1–2 weeks, covered with water. A pale pink solution of phycoerythrin is produced.

B 5e. Hang some *Fucus* (sea-wrack) in a beaker of 70% alcohol for some time. The brown pigment (phycophaein) dissolves out fairly readily, thus leaving the seaweed green with its remaining chlorophyll.

B 5f. Take petals of blue, purple or red flowers (e.g. rose, violet) and extract with boiling alcohol in a flask in a water bath (no exposed flame!). Filter. The alcoholic extract turns red upon addition of a little acid, and blue or green upon addition of a little alkali, with other colour changes upon addition of iron salts, etc.

B 5g. Similarly extract anthocyanin pigments (with water) from beetroot, copper beech leaves, etc. and test the action of acid, alkali and salts.

B 5h. Hold white flowers (e.g. snowdrop, white hawthorn, white lily) over an ammonia bottle. They turn yellow owing to the production of an anthoxanthin pigment, such as occurs in some yellow flowers.

B 5j. Chop up some dock leaves and extract with hot water. Filter, and to the extract add ammonia solution. It turns yellow, owing to reaction with pigments of the anthoxanthin group.

B 5k. Pound 3–4 brown "marble" oak galls in a mortar. Boil the powder well with a little water in an evaporating basin, stand for $\frac{1}{4}$ hour, and filter. The filtrate contains tannin. Dilute a little tannin extract, and add a few drops of dilute ferric chloride solution. A deep blue-black coloration is caused. To another sample of tannin extract add concentrated potassium chromate solution. Tannin is precipitated.

B 5l. Place some grains of "blue" barley (var. *caerulescens*) in a watchglass, and cover with 2% sulphuric acid. A few grains with injured coats turn red, owing to the action of the acid on a litmus-like pigment in the grain. The other grains swell but remain blue, since the coat is permeable to water but not to sulphuric acid. Fix up another set, after pricking the seed coats with a needle. All the grains go red in this case.

See also experiment A 1b.

B 6. Common Foods.

B 6a. Add a few drops of osmic acid solution to bread, potato, cream, butter, broad bean and castor oil seed. Note whether or not the fat-reaction occurs.

B 6b. Crush up various foods (e.g. carrot, apple, grape, bread, potato) separately with water, and filter off the extracts. Test

the filtrates for reducing and non-reducing sugars by means of Fehling's solution.

B 6c. Add a few drops of iodine solution to slices of potato, carrot, bread, apple, and to any other food materials. Note whether or not the starch-reaction occurs.

B 6d. Cut sections of dahlia or Jerusalem artichoke tubers, and place some in water and some in 95% alcohol for an hour. Examine microscopically, when sphaero-crystals of inulin will be seen precipitated in the sections in alcohol.

B 6e. Carry out the Millon's or the Xanthoproteic test upon bread, meat, unboiled white of egg, cheese, rice, lard etc. Note whether or not the protein-reaction occurs.

B 6f. Place some flour in a muslin bag. Knead the flour-bag thoroughly in water in a clean dish. After a while test the water for starch, and the "gluten" remaining in the bag for protein.

B 6g. Halve a potato and scrape the clean surface very finely with a pen-knife. Stir the scrapings well in a beaker of water, and strain through fine muslin. Allow the filtrate to stand, when the starch settles. Decant, add fresh water and repeat lixiviation several times, thus obtaining starch grains fairly pure. Examine microscopically, and also test with iodine solution. Test the first-decanted liquid for protein with Millon's solution.

B 6h. Examine a drop of milk under the microscope and observe the fat globules.

B 6j. Take 5 ml. milk in a test tube nearly filled with distilled water, and add 3 drops concentrated acetic acid. A flocculent precipitate of casein is formed (this carries down fat with it). The precipitate may be tested for protein in the usual way; and for fat by shaking with ether, decanting, evaporating ether by standing the container in hot water (no exposed flame!) and obtaining a grease spot on paper. The filtrate may be tested for lactose with Fehling's solution.

B 6k. Shake grated cheese in a test tube with ether, and decant. Allow the extract to evaporate and test it for fat by obtaining a grease spot on paper. Extract the undissolved part of the cheese by grinding in a mortar with 2% sodium carbonate solution and filtering. Precipitate casein with a few drops of concentrated acetic acid, and test it for protein with Millon's solution.

B 6l. Crush some peanuts and extract with ether. Allow the ether extract to fall drop by drop on to a filter paper, allowing each drop to evaporate before the next falls in the same place. A marked grease spot is obtained.

B 6m. Prepare the ash of various foodstuffs by heating them in a crucible, at first gently and finally on an open flame, stirring to expose the contents to the air. Test for mineral salts as in experiments B 4b–h.

TOPIC C.

THE SOIL

Soil usually consists in the main of rock particles, often encrusted with or adsorbing various mineral salts. In addition there are varying amounts of humus, water (with its dissolved solids and gases) and air.

The differently sized particles may be observed directly under the microscope, and a soil may be analysed into its components either by the use of a series of sieves of varying mesh, or by sedimentation in water (the smallest particles taking longest to settle). The particles form a gradation of size from the very smallest to the very largest, but it is usually convenient to make a conventional division such as:—

gravel - - -	$>$ 2 mm. diameter
coarse sand - -	2 mm.—200 μ diameter
fine sand - - -	200—20 μ diameter
silt - - -	20—2 μ diameter
clay - - -	$<$ 2μ diameter

Rock particles consist mainly of minerals such as silica and complex silicates and alumino-silicates. Those of sand and silt are chemically almost inactive. Clay particles, being of very small size, have colloidal properties, and in addition they are of different crystalline structure, much of their calcium, magnesium, potassium and sodium being fairly easily exchangeable. Thus although normally calcium is the most important base, excessive leaching tends to cause its replacement by hydrogen, with consequent acidity; while incursions of the sea tend to cause replacement by sodium. Treatment with calcium salts is the usual practice with such acid or sodium clays.

The soil moisture is usually from 5–20% of the soil weight, and is distributed in the pore spaces, and in dry soils as a film surrounding the clay particles. It usually contains from 0·05

–0·2% dissolved matter, largely calcium, potassium, nitrate, bicarbonate, sulphate, chloride, and phosphate ions. The agricultural value of a soil is largely determined by its moisture-properties, depending in turn upon the solid soil components. It is believed that the capillary-raising power of soil is severely limited, and of almost negligible significance for the plant.

The micro-organisms of the soil are of vital importance. Bacteria are present which can decompose cellulose, as can some fungi; other bacteria can decompose phenol, cresol etc.; others the proteins and their nitrogenous derivatives; while yet other bacteria and fungi can utilise atmospheric nitrogen. Soil protozoa (rhizopods, flagellates and ciliates) are also important, especially as causing "soil sickness". Altogether a rich soil may contain a flora and fauna of as many as 10^8 individuals per gram.

Humus is formed by the action of micro-organisms on cellulose etc. in plant remains, by the breakdown of the lignin of plants, and also from the mycelia of soil fungi. It has colloidal properties including great water-absorbing power.

The physical and agricultural properties of the soil are a function of its qualitative and quantitative composition. Small particles cause retention of and relative impermeability to water, while large particles have the reverse effect. The mineral salts present are of great importance as food for crops, while the humus is important both for its effect on the physical condition of the soil, and as food material for the living organisms of the soil. These in turn (ranging from bacteria to earthworms and even larger animals) constitute a biotic factor of importance in the growth of crops.

The elementary study of soil involves few theoretical difficulties, and should be closely linked with work in the school garden and various natural ecological habitats. With its physical, chemical, geological and biological inter-relationships, it provides a particularly good introduction to a course in General Science. Many simple and easily performed experiments are available.

Experiments are classified as follows:—

C1. Constituents.
C2. Physical Properties.
C3. Cultivation.
C4. Living Organisms.

C 1. Constituents.

C 1a. Mount small pinches of sandy, clayey and loamy soil, on separate microscope slides. Examine with the low power of a microscope in strong reflected light, observing differing sizes of the particles.

C 1b. Place soil in a tall cylinder to a depth of about 4″, and shake up vigorously with water containing Na_2CO_3. Allow to settle. Examine after a few minutes, and also daily. Gravel, coarse sand and fine sand settle almost at once. Silt and clay settle slowly. Very fine clay particles may still be suspended after a week. Plant and animal debris may remain floating on the top of the water.

C 1c. Dry some soil by leaving it spread out on paper exposed to the air for some days, until it is thoroughly loose in texture. Now shake and rub the soil through a tower of sieves of various meshes, with the widest mesh at the top and the finest at the bottom. Weigh the contents of the various sections, thus obtaining a quantitative analysis of the soil.

C 1d. Bare a portion of soil by a clean horizontal spade cut. Invert a circular tobacco tin (of known capacity v) on the soil, and by a screwing motion press it in until the tin bottom is flush with the soil surface. Dig the tin out and cut the soil off level with the tin rim. Empty the soil into 200 ml. water in a measuring cylinder, shake, allow to settle and read water level. It will be less than $(200+v)$ ml. by the volume of the air in the soil.

C 1e. Add 100 ml. of 20 vol. (6%) hydrogen peroxide to 10 gm. air-dry soil in a litre beaker. Warm slightly, when

vigorous frothing sets in, owing to reaction between humus and the peroxide. Add 200 ml. water when the reaction appears complete, stir, wash down the sides of the beaker with water, and allow to settle. The soil appears much paler owing to the loss of humus.

C 1f. To samples of soil add a few drops of dilute hydrochloric acid. If the soil contains an appreciable amount of calcium carbonate there will be effervescence, due to the evolution of carbon dioxide. In this event a drop of lime water held on a glass rod above the effervescence turns milky.

C 1g. Shake up some soil with distilled water, allow to settle, and filter several times until free from fine clay particles. Evaporate the solution considerably, and test for salts as in experiments B 4b–h.

C 1h. Repeat experiments C 1b–e with different types of soil (e.g. clay, sand, loam, peat), comparing the results.

C 2. Physical Properties.

C 2a. Place loose plugs of cotton wool in each of three filter funnels standing in beakers. Half fill the funnels with equal volumes of sandy, loamy and clayey soils. Pour on to them equal volumes of water, and measure the times required for them to filter through, thus comparing the soils' permeabilities to water. (*N.B.*—Pack soils equally tightly.)

C 2b. Fill three burettes with water, and to the top of each attach a glass funnel by means of rubber tubing. In each funnel place cotton wool, and then fill up with sandy, clayey and loamy soils respectively. Open the taps and compare the rates at which water leaves them, thus comparing the soils' permeabilities to air. (*N.B.*—As in C 2a.)

C 2c. Place equal volumes of well packed air-dry sandy, clayey and loamy soils on cotton wool in filter funnels. On to them pour equal measured volumes of water, and catch in measuring cylinders the water passing through. When drip-

ping has ceased, compare the volumes of water which have passed through and hence by subtraction the volumes of water absorbed by the three soils. (*N.B.*—As in C 2a.)

C 2d. Fill three beakers with equal volumes of moist sandy, clayey and loamy soils, and weigh daily until there is no further loss of weight. Represent the results graphically, thus portraying the relative water-retaining capacities of the soils.

C 2e. Fill three glass tubes with equal volumes of dried sandy, clayey and loamy soils, covering the lower ends with muslin. Stand the tubes in about 1″ of water in a big trough. Observe the heights to which the water rises in the tubes, thus comparing the relative capillary-raising powers of the soils, making sure that the water has ceased rising. (*N.B.*—As in C 2a.)

C 2f. Experiment C 2e can be made more interesting by plotting the height to which the water has risen in each soil, against time. It is usually wise to record the height every minute for the first ten minutes, then about every five minutes and finally at suitable longer intervals until the water ceases rising. The water rises quickly in sand, but soon reaches its maximum height. In clay it rises more slowly, but has a higher maximum. Observe the times taken to germinate by small dry seeds placed on the top of the soils.

C 2g. Fill a long tube with soil, and pour on to the top water in which a red dye (e.g. eosin or red ink) has been dissolved. After percolating through the soil, the water will be clear, the dye being retained by adsorption by the soil.

C 2h. Bore holes just large enough to admit thermometer bulbs in the side of a wooden box about 6″ deep, at 1″, 3″, 5″ from its top. Fill up the box with fairly dry sandy soil. Read the thermometers, and then place on top of the soil a massive iron plate which has been heated for some time in boiling water. Cover with a sheet of asbestos, and read the thermometers every five minutes for 1–1½ hours. Plot graphically the results for each. The iron acts as an "artificial sun", and the graphs

indicate the penetration of heat to different depths in the soil.

C 2j. Using the "soil heat box" of experiment C 2h, remove the three thermometers, and leave a hot iron on the surface for an hour, covering with an asbestos sheet to prevent loss of heat. Support a thermometer 1″ above the soil, and then remove the iron. Read the thermometer every minute for the first 10 minutes, then each 5 minutes for another 50. The hot soil represents the earth after midday sun, and the graph of thermometer readings against time indicates the radiation behaviour of the earth. (*N.B.*—Protect from draughts.)

C 2k. Repeat experiments C 2h and C 2j, but with two separate boxes, or a box divided into two compartments, one filled with whitish (chalky) soil, and the other with blackish (sooty) soil. Thus record the rates of absorption and radiation of heat by the soils. The darker gives the greater rate in both cases.

C 2l. Fill two metal tins with equal volumes of sandy and clayey soils respectively. Insert thermometers in each and place in a water bath. Heat the water bath, and read the thermometers every minute, plotting the results graphically. After a while, remove the tins from the water bath, and plot the graphs of falling temperatures. The sandy soil both rises and falls in temperature the more readily.

C 3. Cultivation.

C 3a. Fill two flat plant pots with equal volumes of fine soil, having wicks passing through the plant pot holes into water contained in jam jars on which the plant pots are placed. Leave for a week or fortnight, "hoeing" one soil-surface daily with a table-fork. From the water levels in the two jars estimate the value of the common practice of hoeing in reducing water loss from the soil surface.

C 3b. Repeat experiment C 3a, but instead of hoeing, "mulch" by placing leaf mould on one soil surface. The mulched soil loses less water than the untreated soil.

C 3c. Shake up some clayey soil with water, and allow it to stand, observing how the clay particles remain in suspension. Carry out a parallel experiment, using lime water, and observe the effect of the lime in flocculating the clay.

C 3d. Place equal volumes of clay in two funnels, and press well down. On one clay surface sprinkle some powdered lime. Into each funnel pour water. It gradually filters through the limed soil (as a result of flocculation) but not through the ordinary clay.

C 3e. Grow bean seeds against the front of two glass-sided boxes, one filled with loam, the other having tightly packed clay below the top three inches. Water each well, and observe growth, the glass being usually covered by black paper or cloth. The roots in the first box grow uniformly; but in the second they tend to remain in the upper layer, and owing to the water-logging, are comparatively small, and the plant does not thrive.

C 3f. Test some filtered liquid farmyard manure for various mineral salts, as in experiments B 4b–h.

C 3g. Prepare some ash by igniting solid farmyard manure, and test for mineral salts as in experiments B 4b–h.

C 3h. Test various artificial fertilisers for mineral salts as in experiments B 4b–h. (If the fertiliser will not dissolve completely in water, try dilute hydrochloric and nitric acids and if necessary concentrated sulphuric acid.)

C 4. Living Organisms.

C 4a. Make a "wormery" (a box about 2′ high, with base about 2′ by 2″ and with one broad side of glass, with a moveable opaque cover of cloth or metal). Fill with soil, cover with leaf mould, and keep moist. Add several earthworms, and leave for a period with the glass shielded by the cover. Worms burrow in the soil, and when the cover is removed, worm burrows may be seen against the glass.

C 4b. Set up a wormery with layers of different soils (e.g. sandy, loamy, chalky, clayey, gravelly, with leaf mould on top). After a time, worms mix up the different layers, largely as a result of feeding and voiding.

C 4c. Set up a wormery and on the soil surface lay fresh leaves, partly decayed leaves, seeds, feather fragments, wool, small pebbles, etc. and note which the earthworms remove and use in plugging up the entrances to their burrows.

C 4d. Collect the worm casts from a known area of soil which had been cleared a known period earlier. Dry and weigh, and thus get a quantitative estimate of the activity of earthworms in turning over the soil.

C 4e. Fill a long glass tube (at least 2′ long) with rich loamy soil. Allow very dilute ammonia solution to percolate through it. Collect the filtrate in a beaker, and pass through the soil several times, until it no longer smells of ammonia. Test the liquid before and after the experiment for nitrates ("brown-ring" test). The second test is positive. The experiment demonstrates the activity of nitrefying bacteria in the soil.

C 4f. In a conical flask place milk to the depth of 1″. Boil for a few minutes, and allow to cool, after plugging the neck with flamed cotton wool. Drop in a little rich garden soil (holding the neck of the flask above a flame to ensure that no bacteria enter from the air) and swirl the flask to mix the contents. Leave for some days in a warm place. The milk goes sour. Carry out a control experiment, using soil which has been baked. There is no souring in this case, since baking kills soil micro-organisms.

C 4g. Dissolve 0·67 gm. dipotassium hydrogen phosphate and 0·33 gm. potassium dihydrogen phosphate in 1 litre distilled water. This makes a neutral potassium phosphate solution. Now take some air-dry garden soil, and pass it through a coarse wire sieve. To about 100 gm. soil add 1 gm. mannitol (or 5 gm. powdered starch). Mix soil + mannitol into a stiff

paste with phosphate solution. Place into several small crucibles and smooth off the tops with a glass slide. Set up control crucibles with pastes of soil and phosphate solution (without mannitol), and of soil+mannitol and water (without phosphate). Cover the crucibles with inverted beakers and keep in a warm place. In about a week, colonies of *Azotobacter chroococcum* appear on the surface of the soil of the first set of crucibles, its growth being encouraged by the foodstuffs supplied. It grows on the surface, being aerobic. Other soil-bacteria do not multiply, since no nitrogen has been provided in the nutrient solution. If the soil is acid, add a little chalk powder. If the experiment fails, repeat with different samples of soil, especially from a fertile garden or a pond bottom.

C 4h. Place some soil in a petri dish, cover with water to the depth of ½″ and add cress seeds which have been boiled for ¼–½ hour. After two days, transfer the seeds with sterile forceps to a sterile petri dish. Cover with water to the depth of ½″, add a glass lid, and leave in a warm place. The seed mucilage acts as a culture medium. At intervals the seeds should be examined microscopically, when fungi derived from the soil will be seen.

C 4j. Place 100 gm. chopped straw into a conical flask, and add 100 ml. of 38% ammonium carbonate solution. Plug the flask quite loosely with cotton wool, and keep in a warm place, spraying the straw with water at intervals, if necessary to keep it moist. Micro-organisms on the straw cause its gradual humification—a process similar to that which goes on in the soil.

N.B.—The ammonium carbonate provides nitrogen for the micro-organisms.

See also experiments D 1c, h, j, l; D 2a, c; K 2d; P 8g, h.

TOPIC D.

MICRO-ORGANISMS

In most schools it is not possible to do a great deal of microscopic examination. Micro-organisms must therefore be studied mainly in terms of their activities and their appearance when massed together in colonies. The easiest to study are bacteria and fungi.

Bacteria often have a diameter of about 1μ and may be approximately spherical (*Coccus*), rod-shaped (*Baccillus*) or curved (*Spirillum*). No definite nucleus can be distinguished. Many species, either throughout or at one stage of their life-cycle, possess flagella and are motile.

Normally bacteria live on other living organisms (i.e. parasitically) or on dead remains of organisms (i.e. saprophytically), but certain species derive their energy from chemical reactions (i.e. chemosynthetically) such as the oxidation of hydrogen sulphide to sulphur (*Beggiatoa*), of ferrous iron to ferric iron (*Leptothrix*), of ammonia to nitrites (*Nitrosomonas*), or of nitrites to nitrates (*Nitrobacter*). In other species the bacteria live symbiotically with another plant (e.g. *Bacillus radicicola* in legume root-nodules), each species contributing to the welfare of the other.

Reproduction normally is effected by simple division, often every $\frac{1}{4}$–$\frac{1}{2}$ hour. Under conditions of adversity however, the cells may form a resistant coat, and as spores may survive until conditions are more favourable. Such spores are often capable of living through extreme cold, heat and drought.

Bacteria cause many diseases (e.g. diphtheria, tuberculosis, gonorrhoea) in man, and in plants diseases such as carrot-rot. Bacteria are also however, beneficial in many ways. Their action in causing decay of dead organisms, in the nitrogen-cycle of the soil, in the maturing of tobacco, in the digestion of cellulose by many animals, are all interesting examples.

Fungi are of various sizes, but only the smaller types are considered here. Like bacteria, they may be parasites, sapro-

phytes or symbionts, or may obtain energy by breaking down various chemicals.

Of great importance in brewing and baking is yeast (the common species being *Saccharomyces cerevisiae*), which converts glucose to alcohol and carbon dioxide; while related fungi are of importance in pickling. *Aspergillus niger* is used to make citric acid from sugar, while other moulds can convert sugars to aromatic compounds. The characteristic flavours of various cheeses depend upon fungi, as do many processes of economic interest.

Fungal diseases are important both to animals (e.g. ringworm and thrush in humans) and to plants (e.g. "damping-off" of cress seedlings by *Pythium* and "club-root" disease of crucifers by *Plasmodiophora brassicae*).

Experiments are classified as follows:—

D1. Bacteria.
D2. Fungi.

See also the following experiments on other types of micro-organism: G 5j; L 1a, b.

D 1. Bacteria.

D 1a. Titrate 20 ml. fresh milk with decinormal sodium hydroxide solution, thus estimating its acidity. Leave another sample of the same milk exposed to the air for two days, and titrate with the alkali. Increase of acidity is due to bacterial fermentation of lactose into lactic acid. Carry out a control experiment with another sample, boiled for a minute, and protected from later contamination by a tight singed cotton wool stopper.

D 1b. A more ambitious experiment is to take a number of samples, and titrate them at intervals of a couple of hours during two days, plotting the results graphically.

D 1c. Shake up 2 ml. loamy soil with 10 ml. freshly distilled water in a sterile beaker. Dip a sterile platinum wire loop into the mixture, and draw lightly in a streak across a sterile

culture medium in a petri dish, taking all possible precautions to avoid contamination. Place in a warm dark place and examine daily. Clusters of bacteria will appear, and gradually increase in size. Carry out control experiments using distilled water+well baked soil, and distilled water only.

D 1d. Make a similar plate culture of fresh milk, and as controls, of well boiled milk, and of pasteurised milk (i.e. kept at 70° C. for 30 minutes, and then cooled rapidly).

D 1e. Place the legs, wings and entrails of a housefly or bluebottle on an agar culture medium in a petri dish. Leave in a warm, dark place. Bacterial and fungal colonies develop.

D 1f. Slip a housefly or bluebottle for a few seconds under the cover of a petri dish containing an agar culture medium. Leave the dish in a warm, dark place. Bacterial and fungal colonies develop where the insect has walked.

D 1g. Pour some boiling water over soaked peas in a beaker, and stand in a warm room for 1–3 weeks. The water becomes turbid owing to the growth of myriads of mixed saprophytic bacteria, which may be examined microscopically.

D 1h. Lift carefully plants of peas, beans, clovers, vetches etc. Wash the roots free of soil and observe nodules caused by bacteria (*Bacillus radicicola*). Compare with similarly treated plants of buttercup, grass, wheat, etc., which have no nodules.

D 1j. Fill two plant pots with good potting soil, and sterilise one by heating for a day in a steam oven. Sow several beans (broad or runner) in each pot. Irrigate both pots with boiled water. When the plants are nearly 1′ high, de-pot and wash away the soil from the roots. Those grown in sterilised soil will have no root nodules, but the others will.

D 1k. Dissolve about a teaspoonful of phenol (carbolic acid) in a litre of water. Pour into a conical flask to the depth of about 2″–3″. In 9 other similar flasks, place equal volumes of the solution, diluted 2, 4, 8, 16, 32, 64, 128, 256, 512 times

(i.e. each half the concentration of the preceding flask). Now dissolve a teaspoonful of ammonium sulphate and half the volume of potassium phosphate (providing mineral salts for bacterial nutrition) in half a litre of water, and shake a teaspoonful of rich soil with it vigorously. Allow to stand for a few minutes, and decant the turbid liquid from the sediment. Pour about an egg-cupful of turbid liquid into each flask, and plug with cotton wool. In some flasks, the contents will in a few days go brown, and lose the "disinfectant" smell, owing to soil bacteria "feeding" on the phenol. In more and in less concentrated solutions, bacteria do not flourish. Microscopic examination confirms the presence of bacteria.

D 1l. Sterilise six petri dishes, each containing a filter paper and about 0·1 gm. calcium carbonate. Shake about 1 gm. soil in 100 ml. water for 4–5 minutes and add 2 ml. of the suspension to each dish. Add also 5–10 ml. of a culture solution containing 1 gm. dipotassium hydrogen phosphate, 1 gm. sodium nitrate, 0·5 gm. magnesium sulphate and 0·5 gm. potassium chloride per litre of water. This encourages the growth of cellulose-decomposing bacteria from the soil and the filter paper should be noticeably eroded after about 10 days. Recently limed well-manured soil gives the best results.

See also experiments C 4e–g; K 2d.

D 2. Fungi.

D 2a. Sow some cress seeds in a pot of damp soil, and cover with a glass bell jar to keep the atmosphere moist. The seedlings eventually "damp off" i.e. topple over and die. Carry out control experiments with (a) dry soil (b) baked soil moistened with distilled water (c) baked soil moistened with water in which some damped off cress seedlings have been kept. The results indicate that moisture helps the damping off, that sterilisation prevents it, and that it can be conveyed in a carrier such as water. Damping off is caused by infection with the fungus *Pythium*.

D 2b. Tease out a damped off hypocotyl on a slide and examine microscopically. *Pythium* hyphae will be visible.

D 2c. Prepare a pure culture of the *Pythium* hyphae by repeated selection and growth on sterile 1·5% agar, alternating if necessary with potato agar, on which it grows more vigorously. Inoculate moist sterile soil with the hyphae and sow cress seeds. Damping off occurs.

D 2d. Cover a small piece of damp bread with a bell jar to keep it moist. Moulds will develop—probably *Mucor* (a grey mould, developing black "pin heads") and *Penicillium* (a blue-green mould). Examine microscopically, mounting in 90% alcohol or soap solution. Mount some *Mucor* in water and observe the bursting of its sporangia. The bread may also develop *Eurotium* (a green mould) and *Rhizopus* (a brown-black mould). Other suitable culture media are prune jelly, boiled rice, and horse manure.

D 2e. Leave a few dead flies in pond water in a beaker for a few days. Often *Saprolegnia* (the mould which attacks salmon and goldfish) develops. Examine microscopically.

D 2f. Place about 100 ml. modified Pasteur yeast culture medium (R 1e) in each of several large conical flasks, forming a thin layer at the bottom. To one add a piece of baker's yeast, and to others a raisin, a grape, a piece of fig, a piece of date etc. Leave in a warm place, with the necks of the flasks plugged with cotton wool. A film develops on the surface of the liquid, if the material introduced carried natural yeasts. Microscopic examination shows that the film consists of yeast. As a control keep one flask of solution, without introducing any yeast-bearing materials.

D 2g. Similarly culture salt-tolerant yeasts from sauerkraut, salted cucumbers, etc., using to 1 litre of culture medium, 3 gm. sucrose and about 7 gm. salt instead of 75 gm. glucose.

See also experiments C 4h; K 2d; K 4c, d.

TOPIC E.

GERMINATION

The study of germination in the school must be very largely observational. The gradual growth of the plant may be observed, and compared and contrasted in different species. Distinction should be made between monocotyledons, dicotyledons, and polycotyledons (gymnosperms); and between those seeds (non-endospermic, e.g. dwarf bean) in which the stored food is contained within the cotyledons, and those (endospermic, e.g. castor oil seed) in which it is without. Opportunity should also be taken to define the terms "seed" and "fruit", and to insist upon their precise use.

In some seeds the cotyledons are carried above the level of the ground by growth of the hypocotyl (i.e. the portion of the stem below the cotyledons) and begin to photosynthesise. This is known as "epigeal" germination (e.g. dwarf bean, castor-oil seed). In other seeds the cotyledons remain below the ground and by growth of the epicotyl (i.e. portion of the stem above the cotyledons), new photosynthesising leaves are formed. This is known as "hypogeal" germination (e.g. broad bean, pea).

Germination is dependent upon the action of enzymes which break down the stored foods into transportable form. Starches are acted upon by amylases, celluloses by cytases, proteins by proteases, and fats by lipases. The enzymes are able to function only when the seed has absorbed water, normally through its micropyle. Oxygen is necessary for respiration at this period, while suitable conditions of temperature and light are needed. A high concentration of carbon dioxide inhibits germination, but normally the gas can diffuse out from the seed through the wet testa.

Experiments upon the conditions favouring germination may be linked with gardening practice, and germination tests with nursery practice.

Experiments are classified as follows:—

E1. General.
E2. Effect of Water.
E3. Effect of Oxygen.
E4. Effect of Temperature.
E5. Effect of Light.

E 1. General.

E 1a. The germination of seeds may conveniently be observed by planting them between the wall of a gas jar and a roll of blotting paper, the space inside the paper being filled with wet bog moss. (Suitable seeds are bean, pea, onion.)

E 1b. Plant in soil in small pots, tree seeds which germinate fairly readily, such as sycamore, beech, sweet chestnut, horse chestnut and pine. Observe the growth of the seedlings.

E 1c. Fill a saucer with moist sawdust, and place 100 cress seeds on it. Record daily the number of germinations and hence find (a) mean time of germination (b) day on which most seeds germinate. Repeat the experiment with several different kinds of seed.

E 1d. Germinate about 50 wheat grains on a porous saucer standing in water, leaving in darkness for three weeks. Chop up the seedlings, place in a weighed crucible in a steam oven, and dry until the weight remains constant. Similarly find the dry weight of an equal number of ungerminated grains. The seedlings have the smaller dry weight, material having been respired during germination.

E 1e. Test the percentage viability of small seeds kept on wet blotting paper under a bell jar.

See also experiments K 2c, h; K 3a; M 3a.

E 2. Effect of Water.

E 2a. Place a wad of cotton wool in a gas jar, moisten, add a few barley seeds, and apply the lid. Set up a control experi-

ment with dry cotton wool. Only the first set germinates, indicating the need for moisture.

E 2b. Soak a broad bean overnight and then squeeze. Water or bubbles come from thé micropyle.

E 2c. Take twelve broad beans. Paint over the micropyles of six of them with rubber solution and allow to dry. Soak overnight and then place on wet cotton wool to germinate. The six seeds whose micropyles have been stopped, do not germinate, since they cannot absorb the necessary water (except for a little through the seed coat).

E 2d. Fill a glass screw-top bottle with dried peas dropped into water, and screw in the top tightly. Leave it inside a strong box or in a cupboard. The peas will swell owing to absorption of water, and will shatter the bottle.

E 2e. Stuff dried peas into a match box, and stand it in a saucer of water. Some hours later the box bursts owing to swelling of the seeds.

E 2f. Take a batch of twenty peas. Weigh while dry, and then place to soak in water. At intervals of 6, 12, 18, 24, 30, 36, 42, 48, 54, 60 hours, take out the peas, dry carefully with blotting paper, and weigh. Tabulate the results, and plot a graph of weight against time of soaking. Calculate in each case the percentage increase over dry weight, and plot this against time of soaking. It rises to an asymptote at about 120%.

E 2g. Place some ordinary dried peas in a perfectly dry bottle. Stopper tightly, and place in a water oven for a few hours. Remove and allow to cool. Moisture collects on the bottle's inner surface, showing that even "dry" seeds contain water. The moisture may be tested with anhydrous copper sulphate, which turns blue with water. Use a bottle without peas as a control.

E 2h. Place 10 dried peas in each of 4 muslin bags, allowing plenty of spare room. Soak two sets in water for 24 hours.

Place one dry set and one soaked set in a hot oven for a minute; and one dry set and one soaked set in a refrigerator for a minute. Plant each set, and record the numbers of seeds germinating. The results indicate that dry seeds stand up to extremes of temperature better than do wet ones.

See also experiment A 1k.

E 3. Effect of Oxygen.

E 3a. Place some soaked peas in each of three flasks, and fill with oxygen, nitrogen, and carbon dioxide respectively. The seeds germinate in oxygen, but not in the other gases.

E 3b. Two-thirds fill a gas jar with water which has been recently boiled and will therefore contain no dissolved gases. Pin ten peas at intervals on a stick, and place in the water so that some peas are just at the surface. Keep in a fairly warm place for a few days. The peas near the surface germinate, but those near the bottom, receiving no oxygen, do not do so.

E 3c. Suspend some wet cotton wool by a wire in a gas jar containing a solution of alkaline pyrogallol, about 20 wheat seeds being sprinkled on the cotton wool. Cover with a vaselined lid and leave for a few days. There is no germination, indicating the need for oxygen, which is absorbed by the pyrogallol. As a control, set up a parallel experiment with water instead of pyrogallol.

E 3d. In each of two large beakers in a sink, place 12 soaked barley grains, covering with inverted wide-mouthed funnels and filling the beakers with boiled and cooled water. Pass a slow stream of water from the tap down one funnel, so that the seeds below it are supplied with aerated water. After a week, the aerated seeds will have germinated much more fully than the others.

E 3e. In each of two large beakers place 12 soaked barley grains. Aerate one with an aquarium aerator. Compare the results after a week as in experiment E 3d.

See also **experiment K 1b.**

E 4. Effect of Temperature.

E 4a. Suspend some wet cotton wool in each of two gas jars, by means of wires, about 20 wheat grains being sprinkled on each piece. Cover with vaselined lids, and leave one jar in a warm place (e.g. over a radiator) and one in a cold place (e.g. a freezing mixture). Only the grains kept in the warm place germinate, indicating the need for warmth.

E 4b. Place 100 cress seeds on each of eight saucers of moist sawdust. Keep moist at temperatures of 0°, 10°, 20°, 30°, 40°, 50°, 60°, 70° C. respectively and record daily the numbers of seeds germinated, thus observing the effect of temperature.

E 5. Effect of Light.

E 5a. Fill two saucers with moist sand. Place 100 onion seeds on each saucer, and place one in a bright light and the other in a dark box or cupboard. Record daily the number of seeds germinating in each saucer. Those in the dark germinate the more rapidly.

E 5b. Repeat experiment E 5a with seeds of willow herb (*Epilobium hirsutum*), tobacco (*Nicotiana tabaccum*), or buttercup (*Ranunculus sceleratus*). In these species, light inhibits germination.

E 5c. Germinate various seeds in light and in dark, and leave the seedlings for some time. Note the etiolation and failure to produce chlorophyll in the dark.

TOPIC F.

CARBON ASSIMILATION

As the word implies, "photosynthesis" is the process of building up chemical compounds by the aid of light. Since not all stages of the process of synthesising starch require light, perhaps a better term is "carbon assimilation".

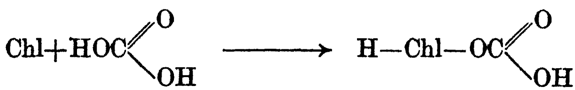
The chlorophyll in the leaf absorbs light especially from the red and blue regions of the spectrum. Thus the reflected light has largely lost these colours, and leaves are green. Magnesium enters into the composition of chlorophyll, and iron is needed for the process of its formation. Part of the energy absorbed from the light is used in the making of hexose and oxygen from carbon dioxide and water. This is a rather complex process, but the net result can be roughly represented by the following equation:—

carbon dioxide + water + light energy $\xrightarrow[\text{aid of chlorophyll}]{\text{with the}}$ hexose + oxygen

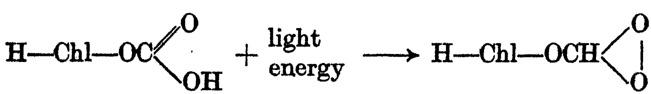
$6CO_2$ $\quad$ $6H_2O$ $\qquad\qquad\qquad\qquad$ $C_6H_{12}O_6$ $\quad$ $6O_2$

Little is known about the mechanism of sugar formation, but one theory is as follows:—

The chlorophyll combines with the carbonic acid (solution of carbon dioxide in water), forming an addition compound:—



This addition compound is then activated by light, being converted into a less stable form, known as "chlorophyll formaldehyde peroxide", which has greater intrinsic energy:—



According to this theory, the chlorophyll formaldehyde peroxide then breaks down, reforming chlorophyll, liberating oxygen, and forming formaldehyde:—

$$\mathrm{H{-}Chl{-}OCH}\Big\langle\begin{matrix}\mathrm{O}\\|\\\mathrm{O}\end{matrix} \longrightarrow \mathrm{Chl} + \mathrm{O_2} + \mathrm{CH_2O}$$

Six molecules of formaldehyde then combine to form hexose:—

$$\mathrm{6CH_2O} \longrightarrow \mathrm{C_6H_{12}O_6}$$

The first-formed sugar is readily converted to other sugars, starches, proteins, fats, etc. These chemical processes do not need light energy and are hastened by warmth in the usual way. The change to starch can be roughly represented by the following equation:—

$$\underset{\mathrm{n\,C_6H_{12}O_6}}{\text{sugar}} \xrightarrow[\text{stages}]{\text{by several}} \underset{\mathrm{(C_6H_{10}O_5)n}}{\text{starch}} + \underset{\mathrm{n\,H_2O}}{\text{water}}$$

The conversion of hexose to starch depends upon the actual sugar concentration, and the "critical concentration" which leads to the formation of starch varies from plant to plant. Thus some plants (e.g. onion), do not normally form starch at all. The reverse process of change of starch to sugar depends upon the action of an enzyme (diastase).

The rate of carbon assimilation depends upon external factors such as temperature, light intensity, carbon dioxide concentration, etc. It used to be held that under any specific set of circumstances, one of these factors is "limiting", and that for increased assimilation, the value of this "limiting factor" must be increased. Recent work, however, has cast doubt on this interpretation, but as a rough guiding principle the theory is still valuable. Assimilation rate is also affected by internal factors such as chlorophyll content, degree of stomatal opening, and inherent diurnal or seasonal rhythms of the plant metabolism.

Experiments are classified as follows:—

F1. Need for Light.
F2. Need for Carbon Dioxide.
F3. Chlorophyll.
F4. Production of Oxygen.
F5. Stomata.

See also experiments B 2q–s; M 2f.

F 1. Need for Light.

F 1a. Half fill a test tube with silver nitrate solution, and add some sodium chloride solution. Allow the precipitate of silver chloride to settle, and then place the tube in the beam of an arc lamp or in bright sunlight. Set up a control experiment, keeping the tube in the dark. Observe the two tubes every few minutes. The precipitate exposed to light gradually darkens—a photochemical action.

F 1b. A similar experiment can be carried out with the precipitate of mercuric oxalate produced by 5% mercuric chloride and 4% ammonium oxalate solutions. This precipitate is much more sensitive and must be prepared in a darkroom. The experiment may be made quantitative by exposing ten tubes to light for periods of 1, 2, 5, 10, 20, 40 seconds and 1, 2, 5, 10 minutes respectively.

F 1c. Keep a potted plant, e.g. clover or young hop or nasturtium (*Tropaeolum*) in a dark cupboard for a day. Test a couple of leaves as in experiment B 2q to ensure that they have been destarched.

F 1d. Destarch individual leaves on a plant shoot in water, by completely enclosing them in light-proof envelopes of black paper for a day. Test as in experiment B 2q.

F 1e. Place a plant with destarched leaves in bright light for some hours. Test leaves for starch. The test gives a positive result. Carry out a control experiment with a plant which has been kept in the dark.

N.B.—Some plants (e.g. onion) do not produce starch.

F 1f. Destarch the leaves of a potted plant (e.g. runner bean). Cover an attached leaf with a tin-foil envelope in which a stencilled word or pattern has been cut. Leave in sunlight for a day, and test the leaf for starch just after midday. The stencilled area appears blue-black. With care, a photographic negative will give a print on a smooth leaf (e.g. lime).

F 1g. Decolorise a filament of *Spirogyra* with alcohol and stain with iodine solution. The resulting coloration indicates the presence of starch around the pyrenoids. Repeat with filaments first kept in the dark.

F 1h. The formation of starch in the light may also be demonstrated by placing leaves (e.g. *Elodea*, moss) in Schimper's solution (chloral hydrate+iodine). The chloral hydrate clears the preparation and the iodine stains the starch, which may be well observed microscopically in the chloroplasts.

F 1j. Bring destarched plants into the light, and remove comparable leaves (e.g. the *n*th leaf from the apex of several privet twigs) at intervals, testing for starch. Once a faint trace is detected, remove a leaf every 5 or 10 minutes, and thus obtain a graded series of leaves of increasing starch content.

F 1k. Sterilise a beaker with formalin, and rinse with boiling water. Half fill with 3% glucose solution. Float on the surface a freshly cut destarched leaf or destarched *Elodea* plant. Put in the dark for a week, and test for starch. The result is positive, indicating that light is not necessary for the conversion of glucose to starch. Carry out a control experiment with distilled water instead of glucose solution.

F 1l. Stand two destarched potted plants in opaque boxes. Cover with dishes containing deeply coloured solutions of potassium dichromate and ammoniacal copper sulphate respectively. Expose to diffuse light for two days, and then test leaves for starch just after midday. The leaves which have received mainly blue light will probably be almost starchless.

N.B.—This experiment fails to make allowance for the relative light *intensities*.

F 1m. Repeat experiment F 4c with different light intensities and differently coloured lights and compare the results.

See also experiments F 3d–f, l; F 4b.

F 2. Need for Carbon Dioxide.

F 2a. Destarch the leaves of a potted plant. Place one attached leaf in a tilted flask containing concentrated sodium hydroxide solution to remove carbon dioxide from the air. Hold the leaf in place by means of a cork split in two, with a channel for the petiole, making joint airtight. Place in sunlight for a day and test the leaf for starch just after midday. The test is negative owing to the lack of carbon dioxide. Almond and white stock are suitable plants.

F 2b. Destarch the leaves of two similar sprigs of mint, keeping the plants in culture solution in a dark cupboard for a week. Place each in its solution in a bell jar on a vaselined glass plate, and by means of an aspirator pass air through each bell jar. In the first case pass the air before entering the bell jars through a flask of "soda water" to ensure that it contains plenty of carbon dioxide, and in the other through a flask of potash solution to remove its carbon dioxide. Leave in bright light for some hours, and then test leaves of the two sprigs for starch. The second sprig gives no reaction, indicating that carbon dioxide is necessary for photosynthesis.

F 2c. Repeat experiment F 4c using several sets of apparatus with (a) pond water (b) recently distilled water, boiled and cooled (c) CO_2—saturated water (e.g. "soda water") and compare the amounts of gas liberated.

F 2d. Repeat experiment F 4c using sodium bicarbonate solutions of various concentrations, and compare the results.

N.B.—Sodium bicarbonate produces the same effective ion as carbon dioxide—about 0·1% to 1% is a useful range.

See also experiments F 5h, j; M 5f.

F 3. Chlorophyll.

F 3a. Draw a variegated leaf (e.g. maple) noting the areas

containing chlorophyll. Test the leaf for starch after it has been exposed to light for some time, still attached to plant. The test gives a positive result only where the leaf was green.

F 3b. Observe microscopically the chloroplasts in a moss leaf, clearing by mounting in chloral hydrate solution. Then mount another leaf in Schimper's solution, and observe microscopically. Starch is stained by the iodine in the solution, and is confined to the chloroplasts.

F 3c. Repeat experiment F 3b with sections of leaves of cherry laurel, etc.

F 3d. Prepare two saucers of damp sand, each with 100 cress seeds. Place one in a light and one in a dark place, and leave for a fortnight, watering regularly. The plants in the dark are etiolated, with no development of chlorophyll.

F 3e. Grow seedlings of mustard (*Sinapis alba*) in the dark, and then place near a window in good light. A distinct green colour becomes visible, often after one hour, owing to the formation of chlorophyll.

F 3f. Sow seeds of pine and of garden pea, and keep for 3–4 weeks in a warm dark cupboard. Although the pea seedlings are colourless, those of pine are green, indicating that light is not necessary for chlorophyll formation in this species.

F 3g. Produce chlorotic plants by growing very small seeds in iron-deficient culture solutions. To some solutions add a few drops of ferric chloride solution. Chlorophyll is formed after a short time. Paint the leaves of some other plants with very dilute ferric chloride solution as an alternative method. It is necessary to use several plants to allow for failures.

F 3h. Repeat experiment F 3g (first part) with a culture solution deficient in magnesium.

F 3j. Grow a bean seedling in the dark, so that it does not develop chlorophyll. Test the capacity of the leaves for

forming starch from the atmosphere (experiment F 1e) and from sugar solution (F 1k in the dark). They cannot do the former, but can do the latter. Thus chlorophyll is necessary only for the first stage of carbon assimilation.

N.B.—There is some recent evidence that chlorophyll *may be* involved in the later stages.

F 3k. Dry nettle powder completely in an oven at 30–40° C. and then in a desiccator. Place small quantities in test tubes, and add pure water-free acetone, ether, benzene, etc. No chlorophyll dissolves out. Repeat with powder moistened by water. The solvents are immediately coloured, the water helping to alter the normal state of the chlorophyll, thus rendering it soluble.

F 3l. Observe the complete spectrum through a direct-vision spectroscope. Place in the path of the light a bottle of chlorophyll-in-acetone. Some wavelengths (red and blue) are preferentially absorbed by the chlorophyll, giving dark bands in the spectrum. As a control, repeat with a bottle of acetone only.

See also experiments A 1b; B 5a–e; E 5c; F 1g, h.

F 4. Production of Oxygen.

F 4a. Shield a potted plant from a lighted candle, and cover both with a stoppered bell jar, making it air-tight. After the flame has expired, place the whole in a bright light for a day. Remove stopper, and apply a lighted taper to the candle. It will burn again, owing to the production of oxygen during photosynthesis. Set up a control experiment in the dark.

F 4b. Place some *Elodea canadensis* or water cress under a beehive shelf in pond water in a larger beaker. Cover with an inverted funnel. Invert over the funnel a test tube filled with water. Leave for a couple of days in sunlight. A gas collects in the test tube, which the glowing splint test indicates to be rich in oxygen. Set up a control experiment in the dark.

F 4c. A very convenient apparatus for measuring the rate of photosynthesis is made as follows. Fix the top of a water

plant stem (e.g. *Elodea*) into one end of a glass tube of approximately the same bore, sealing the joint by means of wax mixture (this is rather difficult). The glass tube should be narrowed at the other end, providing a bubbler through which the oxygen produced during photosynthesis can escape. To ensure that the bubbles are of uniform size, it is wise to pass the bubbler through a bung into a wide glass tube containing oxygen-saturated distilled water. The whole device is then placed in a large vessel of water, in which the shoot photosynthesises. The number of bubbles produced per minute is taken to be proportional to the rate of photosynthesis.

F 4d. Place 100 ml. of 0·075% indigo carmine solution in each of two flasks. Add to each a freshly prepared 7·5% sodium bisulphite solution, drop by drop until the blue colour is just discharged, adding then one drop in excess. In each flask place some waterweed (e.g. *Elodea*) and place one in the dark and one in bright sunshine. In about ½ hour, the latter solution regains its blue coloration, owing to the photosynthetic liberation of oxygen.

F 4e. Remove the oxygen from some defibrinated blood (H 3e) by means of a vacuum pump. Mount a leaf of *Elodea* in a drop of blood on a slide, and cover with a glass slip supported on two other slips to avoid crushing. Observe under the low power of a microscope while exposed to light. The oxygen evolved is made very evident by the gradual reddening of the blood, haemoglobin being converted to oxyhaemoglobin. Set up a control experiment without any green leaf.

F 5. Stomata.

F 5a. Stomata can be well observed in the epidermis of an iris leaf, stripped by means of a razor. Mount in water on a slide, and apply a cover slip. Examine microscopically.

N.B.—Do not place in alcohol, or the chlorophyll will be dissolved out.

F 5b. Boil a privet leaf for a few seconds in very dilute sodium hydroxide solution. Cool and remove a little of the lower

epidermis by means of a razor. Mount in water and examine microscopically. Note the stomata, and compare with a slide of the upper epidermis, which has few or none.

F 5c. Melt a little paraffin wax on a slide and allow it to cool When it is not yet quite hard, press a leaf (e.g. ivy) on to it. Remove the leaf after a little while, and examine the wax microscopically. Impressions of stomata should be visible.

F 5d. Blow into the stalk of a buttercup leaf with its blade dipping into water. Bubbles appear scattered over the surface of the leaf, owing to the forcing out of air through the stomata.

F 5e. Push the end of a piece of glass tubing into the cavity of an onion leaf. Immerse the leaf in water, and blow gently down the tube. Air is forced out of the stomata, causing bubbles.

F 5f. Place a laurel leaf in hot water. The heat causes air to expand and escape from the stomata, producing little bubbles. It is best to coat the cut end of the petiole with vaseline. This experiment provides a very rough method of comparing the numbers of stomata on the two sides of the leaf.

F 5g. Cut a twig of cherry laurel bearing 4 or 5 leaves. Pass its cut end through a hole in a rubber bung in a filter flask, making sure that the joint is air-tight. Fill the flask with tap water, well covering the base of the shoot, and connect to a filter pump. Turn on the pump, when bubbles of air will appear at the base of the shoot. Alter the power of the pump so that the bubbles appear at an easily measurable rate (they will be made more regular by attaching a bubbler as in F 4c with wire and pressure tubing to the base of the shoot). Measure the mean rate over several periods of one minute. Now immerse the leaves in water, and remeasure the rate of bubbling at intervals. Owing to the blocking of the stomata by water, the rate is considerably reduced, air being drawn out of the internal spaces of the plant. Eventually dark patches in the leaves indicate flooding of the air spaces by water.

F 5h. Destarch the leaves of a potted plant. Smear one half of some leaves on both surfaces with vaseline, and then leave the plant in the light for a day. Test vaselined and untreated halves for starch, after removing vaseline. Only the latter give a positive test, since in the former the stomata were blocked up and no carbon dioxide could enter.

F 5j. Destarch the leaves of a potted plant. Vaseline some on their upper, and others on their lower surfaces. Leave the plant for some hours in bright sunlight. Wipe off the vaseline, and test the leaves for starch. The first set should give a positive and the latter a negative result. Now make strips of epidermis from the upper and lower surfaces and compare the numbers of stomata. In most plants the former has few or none and the latter has many, indicating a correlation between photosynthetic efficiency and stomatal frequency.

F 5k. Make a strip of leaf epidermis (e.g. Iris, daffodil, hyacinth) and divide into two pieces. Place one in distilled water and the other in concentrated calcium chloride solution for 10 minutes. Transfer the strips to slides, mounting in the same media. Examine microscopically. The stomata in the strip in calcium chloride solution will be closed, owing to withdrawal of water from the guard cells.

See also experiments J 3b–f.

TOPIC G.

ENZYMES

Enzymes are complex colloidal substances produced by living cells, and act as catalysts (i.e. speed up certain chemical reactions). Their nomenclature is complicated by various overlapping systems, but the simplest rule is to add the suffix "-ase" to a stem indicating the substances upon which the enzyme acts. Thus the enzymes acting on starch are "amylases", those acting on proteins are "proteases", on fats "lipases", on urea "ureases", on maltose "maltases", on lactose "lactases", on sucrose "sucrases", etc.

Often also this suffix is added to a stem indicating some specific process. Thus enzymes which help oxidation-reduction processes are "oxido-reductases", those which catalyse the decomposition of hydrogen peroxide are "catalases", those inverting cane sugar (sucrose) to a mixture of grape sugar (glucose) and fruit sugar (fructose) are "invertases", and so on. Oxido-reductases may be of several types. If they function by the addition of molecular oxygen they are "oxygenases", if by the transfer of oxygen from hydrogen peroxide they are "peroxidases", and if by the removal of hydrogen "dehydrogenases".

In many cases names have been given to enzymes (or mixtures of enzymes) which do not fit into this scheme of nomenclature. Thus the amylase of saliva is called "ptyalin", while that extracted from barley, etc. is called "diastase". The milk-curdling enzyme from cow's stomach is called "rennin", the enzymes produced by yeast are called "zymin", while artificial stomach juice (containing the protease "pepsin") is called "liquor pepticus" and artificial intestine juice (containing an amylase "amylopsin", a lipase "steapsin" and the proteases "trypsin" and "erepsin") is called "liquor pancreaticus". Thus a single enzyme or enzyme-complex may have a variety of names.

Enzymes are very powerful in their catalytic action, and

their activity is affected by the hydrogen-ion concentration of the solution and by the temperature, being completely abolished by boiling. They are also activated by small traces of some chemicals and inhibited by poisons. In animals, enzymes are of chief interest for their action in digestion; while in plants they are responsible for the synthesis of fats, starches, etc. in storage organs, and the breakdown of these stored materials at different times.

Enzymes are generally believed, like inorganic catalysts, to be capable of accelerating a chemical reaction equally in either direction. This has been proved to be so in some cases, e.g. lipase speeds up the synthesis of glycerol and oleic acid to triolein, as well as the reverse process of fat-breakdown.

In mammalian digestion, carbohydrates such as starch and glycogen are converted to maltose by the action of ptyalin (salivary amylase), the action continuing for about half an hour in the upper part of the stomach. Ptyalin cannot act in an appreciably acid solution, but it takes some time for the stomach acid to diffuse into the mass of swallowed food. Ptyalin is active only on starch whose grains have been broken (e.g. by cooking), but the amylopsin (pancreatic amylase) met in the intestine is capable of converting even unboiled starch to maltose. Also in the intestinal juice are maltase which converts maltose into glucose, sucrase (invertase) which converts sucrose into glucose and fructose, and lactase which converts lactose into glucose and galactose.

In the stomach, proteins are first acted on by the protease pepsin; the stomach cells also secreting hydrochloric acid, which provides the acid medium necessary for the enzyme's action. Pepsin breaks down the proteins to peptones, but does not carry the process any further. In many animals, and probably in young humans, the stomach also contains rennin, which causes milk to clot. Passing to the intestine, the semi-digested protein enters a less acid medium and is acted on by the pancreatic protease trypsin (formed by the combination of enterokinase with trypsinogen), the peptones being converted to simple polypeptides. Also in the intestinal juice

is another protease, erepsin, which breaks these polypeptides into amino-acids.

Fats are digested by means of lipases in the stomach and intestine. In the stomach very little action takes place, owing to the acid nature of the contents. In the intestine however, the pancreatic lipase (steapsin) is active, the medium being made less acid by the bile. The fatty acids, as soon as they are liberated, are converted into soluble soaps, which in dissolving set up currents that break up the liquid fat globules into a fine emulsion, thus exposing a large surface to enzyme action.

Experiments are classified as follows:—

G1. Amylases.
G2. Proteases.
G3. Lipases.
G4. Other Enzymes.
G5. Conditions of Action.
G6. Methods of Extraction.

G 1. Amylases.

G 1a. Place some starch grains (e.g. of potato or wheat) in each of two watchglasses. To one add diastase solution, and to the other, as control, add water. Cover to reduce evaporation, and keep in a warm place. After 3–4 hours, mount some grains from each watchglass, and examine microscopically. Those which have been subjected to the action of the diastase are corroded (i.e. digested).

G 1b. Half fill a test tube with 0·5% starch solution. Set out 8 watchglasses on a white background, and in each place a drop of dilute iodine solution. To the starch solution add a few drops of a 0·2% solution of diastase (plant amylase). Transfer a drop of the mixture by means of clean dropping tubes to the watchglasses in turn, at intervals of 0, ½, 1, 2, 5, 10, 20, 30 minutes. The colour changes gradually (blue→purple→brown), owing to hydrolysis of the starch (purple is caused by dextrins formed as intermediate products). Carry

out a control experiment with starch solution without diastase.

G 1c. Before and after experiment G 1b, test the solution in the test tube for reducing sugars, by means of Fehling's solution. A red precipitate in the latter case indicates hydrolysis of starch to reducing sugar (maltose).

G 1d. Place a little starch jelly on the tip of the tongue. After a while a sugary taste is noticed, owing to hydrolysis of starch by ptyalin (salivary amylase).

G 1e. Repeat experiments G 1a–c with saliva obtained in copious secretion by chewing india rubber, and washed out of the mouth with luke warm water. Saliva contains salivary amylase.

G 1f. Repeat experiments G 1a–c with liquor pancreaticus, which contains pancreatic amylase (amylopsin).

G 1g. Repeat experiments G 1a–c with amylase solutions which have been boiled. There is no hydrolysis, the enzymes having been inactivated by heating.

G 1h. Insert a small pipette through the oesphagus and into the mill chamber of a living crayfish, thus withdrawing the digestive juice. Repeat with several specimens to secure a fair supply. The juice contains an amylase (test with starch and iodine) and a protease (test with stained fibrin, making slightly alkaline with 1–2 drops 2% sodium carbonate solution).

See also experiments G 5a, f, g; G 6a, b.

G 2. Proteases.

G 2a. Draw egg-white into a glass capillary tube, and coagulate in boiling water. Break into ″ lengths and cover with liquor pepticus, keeping at about 40° C. The egg-white is slowly digested by peptic protease (pepsin).

G 2b. Repeat experiment G 2a with liquor pancreaticus, which contains pancreatic proteases (trypsin and erepsin).

G 2c. Repeat experiments G 2a, b with stained fibrin (R 1u) instead of coagulated egg-white. The stain gradually diffuses out as digestion proceeds.

N.B.—Carmine is extracted by alkali.

G 2d. Repeat experiments G 2a, b with liquor pepticus and liquor pancreaticus which have been boiled. There is no digestion, the enzymes having been inactivated by heating.

G 2e. Place a small strip of hard boiled egg-white in a test tube, and cover with liquor pancreaticus. Add a small crystal of thymol to prevent bacterial growth. Leave in a warm place, and shortly before all solid has dissolved, test a sample of the liquid by the biuret test. A rose coloration indicates the formation of peptone (by the action of trypsin). Repeat the test at intervals of about 6 hours. The rose coloration gradually gets fainter as digestion of the peptone proceeds and amino-acids, etc. are formed by the action of erepsin.

G 2f. Repeat experiment G 2e, using liquor pepticus instead of liquor pancreaticus. Even after some days a rose coloration is still given, since the peptone is not further digested by pepsin.

G 2g. Add the indicated amount of a commercial rennet preparation to some milk. Heat carefully in a water bath to body temperature (do not overheat). On cooling, the milk sets to junket owing to enzymic conversion of casein to para-casein, which forms a precipitate with the calcium of the milk. As a control, repeat with a sample of milk containing no rennet.

G 2h. Repeat experiment G 2g using instead of rennet some liquor pepticus or other pepsin solution. (Some commercial "rennets" actually contain pepsin rather than rennin.)

See also experiments G 5b, c, h; G 6f–j.

G 3. Lipases.

G 3a. Remove the testa from a castor seed, place in a mortar with a little water, and crush. Pour the suspension into a test tube and add litmus solution to give a definite tinge of colour. Keep for a day in a warm place. The litmus becomes redder, owing to breakdown of oil to acids by the action of lipase.

G 3b. Sterilise by boiling, a test tube partly filled with milk. Cool and add 2 ml. lipase solution, 1 ml. phenol red solution and sufficient 2% sodium carbonate solution to give a reddish tinge. Keep the tube in a water bath at 40° C. The solution gradually turns yellow, owing to hydrolysis of the milk fat to fatty acids, by the lipase. Carry out a control experiment without lipase.

G 3c. Repeat experiment G 3b with boiled lipase. There is no colour change, the enzyme having been inactivated by heating.

See also experiments G 5e; G 6k.

G 4. Other Enzymes.

G 4a. Scrub and peel a potato and grind with water in a mortar. Filter, and to part of the filtrate add a few drops of guaiacum tincture. It turns blue, owing to oxidation with the help of potato oxygenase (an oxido-reductase). Carry out a control experiment using boiled potato peel extract. Boiling inactivates the enzyme, and there is no blue coloration.

G 4b. Fill a test tube with distilled water, coloured by ½ ml. dilute methylene blue solution, and add about 1 gm. zymin. Cork, after adding a few drops of toluol as an antiseptic. Keep at 30–40° C. for a couple of hours. Dehydrogenase (an oxido-reductase) in the zymin reduces the methylene blue to a colourless compound. Set up controls, using no zymin and using boiled zymin.

G 4c. Remove as much muscle as possible from a freshly killed frog, avoiding fat and tendon. Cut into small pieces with a razor blade, and shake up 1 gm. with 4 ml. water. Add to 2 ml. 0·03% methylene blue solution and 6 ml. phosphate buffer solution (pH 7.4) in a boiling tube. Stopper the tube loosely, and remove dissolved oxygen from the liquids by connecting with a vacuum pump until bubbles come off freely. Push the stopper home firmly, and keep at about 35° C. Dehydrogenase (an oxido-reductase) from the muscle causes reduction of the methylene blue, which becomes colourless. Set

up a control experiment using 2 ml. water in place of frog muscle suspension.

G 4d. Grate up a little horseradish and grind the gratings with water. Decant, mix liquid with a little hydrogen peroxide solution, and add to guaiacum tincture. It turns blue owing to oxidation by the aid of peroxidase (an oxido-reductase) extracted from the horseradish.

G 4e. Fill a test tube with 10 vol. hydrogen peroxide solution, and add about 1 gm. zymin. Invert in a basin of water. Catalase in the zymin causes evolution of oxygen ($2H_2O_2 \rightarrow 2H_2O + O_2$) and this may be detected by its action in relighting a glowing splint. Set up controls, one without zymin and one with boiled zymin.

G 4f. To a dilute sucrose solution add a little invertase solution and leave in a warm place for $\frac{1}{2}$–1 hour. Test samples of the liquid both before adding the enzyme and after it has been allowed to act, by means of Fehling's solution. The latter test shows the presence of reducing sugar, due to the inversion of sucrose to hexoses. As a control,set up a parallel experiment with boiled invertase solution and one without invertase.

G 4g. Add a pinch of soya bean meal to 2–3 ml. urine in an evaporating dish. Add a few drops of litmus solution, and 1% acetic acid, drop by drop, until litmus is faintly pink. After a while, an enzyme (urease) in the meal converts urea in the urine into ammonium carbonate, turning the litmus blue.

G 4h. Make two fruit jellies, one with tinned and one with fresh pineapple. The latter gradually liquefies, starting near the pieces of fruit, since fresh pineapple contains a gelatine-lysing enzyme which is inactivated in the tinning process.

G 4j. Chop up some cherry laurel leaves, and place in a gas jar. Suspend from the lid some picric acid paper moistened with sodium carbonate solution. The paper turns red, owing

to the action of the enzyme emulsin which splits up a glucoside into glucose, an aldehyde, and hydrocyanic acid. (The paper detects the acid fumes.) Set up a control experiment with intact leaves—in this case the emulsin does not make intimate contact with the glucoside, and there is no action. (Care!—hydrocyanic acid is poisonous.)

See also experiments G 6c–e, l; H 4g.

G 5. Conditions of Action.

G 5a. Test the reaction of saliva to litmus. It is sometimes faintly alkaline, but usually dissolved substances render it slightly acid. Observe inhibiting effect of acid by repeating experiment G 1e after adding a few drops of dilute hydrochloric acid.

G 5b. Place a few small cubes of hard boiled egg-white in each of three small dishes, and cover with equal volumes of dilute pancreatin solution. To each add a small crystal of thymol to prevent bacterial growth. Leave dish A as it is, to dish B add a few drops of dilute hydrochloric acid, and to dish C a pinch of sodium carbonate. Examine at intervals over 2–3 days. Digestion is rapid in dish C (alkaline), slower in dish B (neutral) and completely inhibited in dish A (acid).

G 5c. Repeat experiment G 5b, using liquor pepticus instead of liquor pancreaticus. In this case the enzyme action is most effective in an acid solution.

G 5d. Dissect a freshly killed animal (e.g. frog) and carefully ligature the gut at each end. Remove the gut entire, ligature between different regions (e.g. stomach, duodenum, rectum, etc.) and cut between them. Withdraw liquid from each section of the gut by means of a small pointed pipette, run out on to a waxed slide, mix with equal volume of an indicator (e.g. B.D.H. "Universal" or "4–11"), and draw up into a capillary tube to observe the colour. Thus the pH of the gut contents in different regions may be observed—and

these are the acidities at which the gut enzymes normally function.

G 5e. Add dilute sodium hydroxide solution to olive oil in a test tube, shaking frequently. An emulsion is formed, as happens in the duodenum with the alkaline digestive juice there. This action depends upon the presence of a trace of fatty acid in the oil.

G 5f. Repeat experiment G 1b with test tubes of starch solution and diastase kept at temperatures of 0°, 20°, 30°, 40°, 50°, 60° C., thus investigating the temperature effect.

G 5g. Repeat experiment G 1b with five test tubes and five sets of watchglasses. To the test tubes add amylase solution in the proportions of 1 : 2 : 4 : 8 : 16 and record the times needed in each case for loss of the blue starch coloration. Hence observe the effect of varying quantities of enzyme.

G 5h. Experiments may be devised similar to G 5f (on temperature) and G 5g (on enzyme concentration) using stained fibrin and liquor pepticus and observing the time required for production of a definite tinge of pink in the solution.

G 5j. Allow bacteria to develop in a hay infusion, and add to it a very small amount of neutral red. Feed some of the stained bacteria to *Amoeba*, which ingests some of them. The food vacuole contents are thus stained red, but as digestion of the bacteria proceeds, the contents become alkaline and the vacuole therefore yellow.

See also experiments G 1g; G 2d; G 3c; G 4a, b, e, f, h.

G 6. Methods of Extraction.

G 6a. Germinate 50 barley grains on moist blotting paper for 4–5 days. Place in a mortar, cover with distilled water, and grind. Allow to stand for 2–3 hours and filter. The filtrate contains plant amylase (diastase).

G 6b. Extract a fresh minced fat-free pig's pancreas with twice its weight of pure glycerol, for 12–24 hours at 30–40° C. The solution contains pancreatic amylase (amylopsin).

G 6c. Chop up 2–3 gm. plant tissue and grind with 20 ml. water, 2 ml. toluene, and fine sand. Filter after ½ hour and make filtrate up to 25 ml. with distilled water. The solution contains sucrase (invertase).

G 6d. Grind some yeast with an equal volume of silver sand in a mortar, moistening slightly. Gradually add more water, mixing to a very thin paste. Filter, if possible through a Buchner funnel, repeating if necessary to obtain a clear filtrate which contains yeast sucrase (invertase).

G 6e. Place 20–30 bees in a small glass bottle, and kill with a little ether. Crush in a mortar with 10 ml. distilled water, filter and add 1 drop toluol to prevent bacterial growth. The extract contains sucrase (invertase) from the bees' salivary glands.

G 6f. Scrape off the inner surface of the stomach of a freshly killed pig, mince finely, add some 0·2% hydrochloric acid, and leave in a warm place for a couple of hours. Strain through muslin. The filtrate contains gastric protease (pepsin).

G 6g. Mince the fat-free pancreas of a freshly killed pig and add three times its weight of 0·5% hydrochloric acid. Stir well for ½ hour, add 5% sodium hydroxide until the pH is about 5, and filter. The filtrate contains pancreatic protease (trypsin).

G 6h. Wash out under the tap the contents of the small intestine of a freshly killed pig, split open, and scrape off the interior surface. Grind with sand in a mortar and extract with 1% sodium chloride solution. Filter. (This may take several hours.) The solution contains intestinal peptase (erepsin).

G 6j. Chop up some butterwort leaves with water. The mash contains an enzyme similar to rennin.

G 6k. Weigh the fat-free pancreas of a freshly killed pig, mince and grind with sand in a mortar. Treat with four times its weight of 5% alcohol. After standing for three days strain through muslin. The liquid contains pancreatic lipase (steapsin).

G 6l. Prepare potato juice as in experiment G 4a. Pour into alcohol. A precipitate is obtained, which on separation and solution in water, will not act as an oxygenase (G 4a) but will act as a peroxidase (G 4d). Part of the potato juice dissolves in the alcohol. This part contains phenolic substances which can combine with atmospheric oxygen to give organic peroxides, which then pass on their oxygen to the peroxidase (i.e. potato oxygenase=potato peroxidase+phenolic substances).

N.B.—It is this formation of organic peroxides which is responsible for the "browning" of potatoes, apples, etc.

See also experiments G 1e, h.

TOPIC H.

CIRCULATION IN ANIMALS

The circulatory system of an animal is of prime importance, the more so the larger and more complex the animal. It may be responsible for the transport of foods, dissolved gases, excreted material, hormones, anti-bodies, etc. and aids in the distribution and regulation of heat. In simple animals such as *Amoeba*, the flowing protoplasm acts as a circulatory system, while in rather more complex groups flagellae and cilia may provide the motive agent. In the earthworm and more complex types, there is usually a closed circulation in blood vessels, the motive agent being the rhythmic muscular movement of vessels or hearts. In insects and related groups there is a partly closed and partly open circulation.

The heart has usually two chambers in fish, three in amphibia and reptiles, and four in birds and mammals. Blood leaves the ventricle(s) of the heart by arteries, passes thence along arterioles into capillaries, and back by venules and veins to the auricle(s).

Human blood consists of plasma (90% water and 10% dissolved proteins, sugar, fat products, salts, products of excretory activity, carbon dioxide, enzymes, hormones, etc.), red corpuscles (anucleate, about 7·5 μ in diameter, bi-concave, about 5×10^6 per cubic millimetre), white corpuscles (nucleate, of various sizes and shapes, about 6×10^3 per cubic millimetre), and platelets (about 3×10^5 per cubic millimetre, smaller than red corpuscles). Upon standing it clots, giving a clear serum.

In most animal groups the oxygen-carrying pigment of the blood is haemoglobin (consisting of the iron-bearing group "haem" and the protein group "globin") which is readily convertible to oxyhaemoglobin. In some groups however (e.g. molluscs, crustaceans), this function is carried on by haemocyanin, a substance similar to haemoglobin but containing copper instead of iron.

In addition to the blood circulation there is in many animals a circulation of lymph. This consists of plasma together with a few corpuscles and other components. It bathes the cells of the body, and is always interchanging its constituents by osmosis and diffusion with those of the blood. Lymph is responsible for the transport of fat-products from the villi of the intestine and their eventual transfer to the blood. There is a system of lymph vessels just as there is of blood vessels.

Most school work on circulation will relate to blood, and especially to haemoglobin, but other aspects should not be completely neglected.

Experiments are classified as follows:—

H1. Channels of Circulation.
H2. Heart and Pulse Beat.
H3. Blood Components.
H4. Haemoglobin.

H 1. Channels of Circulation.

H 1a. Take two pieces of glass tubing, each about 1′ long and ½″ in diameter. Insert one into the right auricle of a sheep's heart, and one into the right ventricle, tying firmly with string to the vein and artery through which they are respectively inserted. Tie up with string any other blood vessel openings attached to the heart. Hold up the heart by the glass tubes, and fill with water the tube in the auricle. Rhythmically squeeze the ventricle with the hand. Water sinks in the one tube and rises in the other, demonstrating the one-way action of the heart valves.

H 1b. Roll up the sleeve to the elbow, and tie a handkerchief fairly tightly around the arm just above the elbow. After a while the veins swell with blood, the position of the valves being obvious. Stroke the veins firmly, first towards and then away from the heart. The blood passes the valves in the former case, but accumulates at them in the latter. It is well to select pupils with conspicuous veins for this experiment.

H 1c. Repeat experiment H 1b with the handkerchief tied very tightly. This stops the flow of blood along the more deep-seated arteries, and the fore-arm becomes pale and numb.

H 1d. Examine microscopically by strong reflected light, the very thin skin overlapping the base of a finger nail. (Things are much clearer if a drop of cedarwood oil is first placed on the skin.) Capillaries can be seen, with the blood moving in them.

H 1e. Place a frog for 30 minutes in a 2% urethane solution. When it is anaesthetised, place on a card on a microscope stage and examine the web of the foot microscopically by reflected light, or by light transmitted through a hole in the card. Capillaries and blood circulation can be seen.

H 1f. Place a very young tadpole in a shallow watchglass of water with one drop of chloroform. Examine the external gills microscopically, when the capillaries and blood flow can be seen. Examine also the thin skin of the tail.

H 1g. Circulation can be well observed in young trout just before or just after hatching. The heart beat can be seen with the unaided eye, and capillary flow under the microscope.

H 2. Heart and Pulse Beat.

H 2a. Sit comfortably on a chair with legs crossed. The hanging foot moves rhythmically with the heart beat. By means of hook and thread connecting the shoe with a long light lever, these movements may be magnified so as to be readily visible. Permanent records may be made on moving sooted paper.

H 2b. The class splits into pairs. Each person times the partner's heart beat rate over three periods of two minutes, calculating the mean frequency. The measurement is repeated for the beats of wrist and temple pulses and the three frequencies compared. The partner then undergoes some form of vigorous exercise for five minutes, and the heart and pulse frequencies are again measured. The experiment can be extended by measuring pulse rate over successive two-minute

periods until it returns to normal, and then representing the results graphically.

H 2c. Immediately after a frog has been killed by chloroform, open up its body cavity, removing carefully the connective tissue around heart. The heart will probably begin to beat, and the order of contraction of auricles and ventricle can be observed.

H 2d. Examine microscopically the heart of a *Daphnia* in a compressorium. Count the number of beats per minute, recording also the temperature of the water. Repeat at different temperatures from 0° to 30° C., and plot graphically heart rate against temperature.

H 2e. Place a small tadpole with external gills in a small glass dish and examine microscopically, focussing on the gills and observing pulsation rate. Add drop by drop a concentrated solution of CO_2 in water, and plot pulsation rate against number of drops added. The graph rises until excess produces a toxic effect, and then falls.

See also experiment P 5d.

H 3. Blood Components.

H 3a. Tie a thread tightly around the last joint of a finger, and bend the finger. Sterilise a needle in a flame, and prick the finger just behind the nail. A drop of blood exudes, and when examined microscopically on a warm slide the red corpuscles can be seen, together with some amoeboid white ones.

H 3b. Decapitate a frog quickly with a large pair of scissors. With a fine syringe withdraw a little lymph from the dorsal lymph sac. Mount in Ringer's solution on a warm slide and examine microscopically. The amoeboid white corpuscles may be seen altering in shape.

H 3c. Place a drop of blood (obtained as in H 3a) on a slide and examine microscopically, keeping the slide perfectly still. After about 20 minutes, fibrils may be seen pervading the blood.

H 3d. Place some fresh blood (obtained from a lairage) in a jar, and allow to stand for some time. It clots, leaving a clear yellowish serum (consisting of plasma plus some white corpuscles).

H 3e. Repeat experiment H 3d, but first beat the blood vigorously with birch twigs or shake well in a flask with glass beads. Fibrous material (fibrin) collects on the twigs or beads and the defibrinated blood does not clot. In a clot, fibrin has entangled with it most of the blood corpuscles.

H 3f. Repeat experiment H 3d, but first add to the blood $\frac{1}{10}$th of its volume of 10% sodium citrate solution. The blood does not clot. This action of sodium citrate is used in blood-transfusion practice.

H 3g. Prepare some blood fibrin (experiment H 3e) and test it for proteins as in experiments B 3a–g.

H 3h. Prepare some blood serum (experiment H 3d) and dilute 15 times with distilled water. Cloudiness is due to the precipitation of a protein (serum globulin).

H 3j. Dilute 5 ml. defibrinated blood to 30 ml. with water, and boil. Add 25 ml. water containing 1–2 drops glacial acetic acid, and reboil. Allow the precipitate to settle and filter. Evaporate the filtrate down to about 15 ml. and test for chloride (B 4h), phosphate (B 4e) and sugars (B 2g–p). Heat the precipitate strongly in a crucible, allow to cool, dissolve in dilute hydrochloric acid, and test for iron (B 4d).

See also experiments A 3m, n.

H 4. Haemoglobin.

H 4a. To some very dilute blood, add a few drops of potash solution and warm. The blood turns brown, owing to the haemoglobin's being converted into haematin and globin.

H 4b. Place some fresh blood in a flask and dilute with about twenty times its volume of water. Shake vigorously. A bright

reddish coloration indicates the formation of oxyhaemoglobin by the combination of haemoglobin and oxygen. Now connect the flask with a filter pump and draw off the combined oxygen. A change to a darker purplish colour indicates the re-formation of haemoglobin by the loss of oxygen from oxyhaemoglobin.

H 4c. Repeat experiment H 4b, but reduce the oxyhaemoglobin by adding a few crystals of sodium hydrosulphite instead of drawing off the oxygen by means of a filter pump.

H 4d. Add $\frac{1}{10}$th of its volume of 10% sodium citrate to freshly drawn blood to prevent clotting. Bubble oxygen through it. The blood becomes bright red owing to the formation of oxyhaemoglobin. Now bubble carbon dioxide through it. It becomes purplish, owing to the re-formation of haemoglobin by the loss of oxygen from oxyhaemoglobin. The oxygen leaves the liquid because its partial pressure is less in the bubbles than its effective value in the oxyhaemoglobin.

H 4e. Observe a test tube of diluted fresh blood by means of a direct vision spectroscope. Shake vigorously, and repeat the observation. The absorption bands of the spectrum change, owing to the conversion of haemoglobin to oxyhaemoglobin.

H 4f. Add a drop of blood to water in a test tube, and mix with guaiacum tincture and hydrogen peroxide. A blue coloration is given, since haemoglobin acts as a peroxidase.

N.B.—This is a very delicate test for blood.

H 4g. Bubble a stream of coal gas through diluted defibrinated blood. A cherry red colour develops, owing to the formation of carboxyhaemoglobin (or better—carbonylhaemoglobin).

See also experiment F 4c.

TOPIC J.

CONDUCTION IN PLANTS

Conduction in plants is in some ways rather analogous to circulation in animals. Most of the smaller plants depend largely on simple osmosis and diffusion. The larger plants have more specialised conducting tissue, consisting in the main of long single-celled tracheids and longer tracheae derived from many cells. These woody tissues also have a skeletal function. Some conduction is also effected by the circulation of protoplasm (e.g. *Elodea*, *Tradescantia*).

Experiments are most easily done on terrestrial flowering plants. In most cases water is absorbed by the roots and so are salts in the soil solution. Water enters the root when its cells have a suction pressure greater than the osmotic pressure of the soil solution. In at least some plants, the root system is able to draw water from the soil, but the main motive force of the transpiration stream is the evaporation of water from the leaves, fresh water passing up and taking its place.

Evaporation from most land plants takes place mainly through the stomata, although some occurs through the cuticle, and in some rain forest plants this accounts for over half the total. It is not true that transpiration is necessarily slow in the so-called "xerophytes."

The rate of evaporation of water from land plants depends upon external factors such as humidity, temperature, wind, pressure and light. The total "evaporating power" of the atmosphere may be measured by various types of atmometer, of which the porous pot type is the most popular. Clearly evaporation rate will also depend upon internal factors such as the degree of opening of the stomata and the water content of the leaf mesophyll cells opening to the stomatal space. It seems that the former is important when the stomata are nearly closed, but the latter much more so with fairly widely opened stomata.

The mechanism of stomatal movement is of interest. Light causes the enzymatic hydrolysis of starch to sugar in the

guard cells, with consequent increased osmotic pressure, suction pressure and turgot, leading to opening. This action of light is totally distinct from its photosynthetic effect. Naturally any other factor affecting turgidity (e.g. humidity, temperature) will also affect stomatal opening. Many plants however, have a well marked intrinsic rhythm of stomatal opening and closing, irrespective of external conditions.

An important aspect of the plant conducting system is the transport of synthesised foods. This is a slower process than the movement of water and its dissolved salts in the transpiration stream, and depends upon the vital activities of living cells. It is however, very much too rapid to be explained by any simple diffusion mechanism. Translocation of sugars, etc. in solution, normally takes place in the phloem or "bark vessels". Chemicals are translocated in the form of simple sugars, amino-acids, soaps, etc., and then converted to starches, proteins, fats, etc. by enzymes in the storage organs.

Water absorption is often stated to be of value to the plant in that it causes the absorption of mineral salts from the soil. This is untrue, since salt absorption is a totally distinct process independent of transpiration. Water evaporation is usually believed to be of value to the plant in that it causes cooling and hence prevents excessive heating. In many cases however, this effect seems to be almost negligible. It may be that the transpiration stream is merely a corollary of the presence of stomata, which are necessary to the gas exchange of photosynthesis.

Experiments are classified as follows :—

J1. Water Economy.
J2. Action of Roots.
J3. Action of Leaves.
J4. Channel of Transpiration.
J5. Translocation.

J 1. Water Economy.

J 1a. Moisten the soil around a potted nasturtium (*Tropaeolum*), drain well, and weigh daily. As a control, weigh

daily a similar pot of soil without any plant. Tabulate and graph the results, which show clearly that weight is lost by the plant. This is due to evaporation of water, mainly from the leaves.

J 1b. Experiment J1a can be modified, using a potted plant with the pot and soil encased in a rubber sheet to prevent evaporation except from the shoot.

J 1c. Experiment J1a can be modified, using a plant with its roots in a jar of water, covered with oil to prevent evaporation except from the shoot.

J 1d. As in experiments J 1a–c measure the rates of evaporation from a plant, in sun and in shade, in warmth and in cold, in wind (natural or artificial) and in calm.

J 1e. The potometer is an instrument for measuring the rate of water absorption by a shoot. This is normally assumed to be equal to the rate of evaporation of water from the plant, although the assumption is not always legitimate. Potometers of many types can be quite easily constructed. A convenient one is Thoday's potometer. A clean edge to the shoot is cut (under water, to prevent blockage of the wood by bubbles of air). The cut shoot is now inserted in the rubber tube of the potometer, while the instrument is full of water which will be slowly flowing from it. It is well to tie the rubber tube tightly around the shoot with fine wire. When the clip below the water funnel is closed, the evaporation of water from the leaves leads to the uptake of water by the shoot, and thus to a slow movement of the meniscus along the capillary tube. Normally the absorption rate is calculated by taking the reciprocal of the mean of three successive measurements of the time required for the meniscus to pass between two marks on the scale alongside the capillary tube. After each reading the clip is released for a moment to force back the meniscus to the open end of the capillary tube.

J 1f. With a potometer, measure the absorption rate of a

shoot, in sun and in shade, in warmth and in cold, in wind (natural or artificial) and in calm.

J 1g. Place a potometer with an attached shoot on a sensitive balance in a place well protected from draughts. At intervals throughout a day record absorption rate (by potometer) and evaporation rate (by loss of weight). Record the results in a table and represent graphically, thus obtaining the relationship between the two rates.

J 1h. Measure the rates of transpiration (evaporation and absorption) of bog and salt marsh plants, compared with those of ordinary grassland. Measure also the leaf areas and calculate in each case the transpiration rate per unit leaf area.

J 2. Action of Roots.

J 2a. Nearly fill a test tube with water and place a small plant with its roots dipping into it, the plant being held in position by cotton wool in the neck of the tube. After a few days the water level will have fallen noticeably owing to absorption by the roots. As a control set up a similar tube without any plant.

J 2b. Place a potted fuchsia in a trough, and add sufficient water to flood the soil and rise about 4″ above it. Cut across the stem 2″–3″ above the soil, and quickly attach by means of rubber tubing a glass tube a few feet long. Wire the rubber connection at both ends, to make tight joints. Slowly water rises in the glass tube, by "root pressure". The experiment works best in the spring, and better with some species than with others.

J 2c. Wash carefully the roots of two similar plants (e.g. groundsel). Place one in water coloured with eosin or red ink (a solution) and the other in water coloured to about the same tint with carmine powder (a suspension). Examine after a day. The first plant is coloured within, but the second is not, indicating that roots do not absorb suspended solids.

J 3. Action of Leaves.

J 3a. Place a leafy potted plant (with the soil covered by a sheet of tin foil) under a bell jar and leave in the light. The inside of the jar becomes beaded with moisture, and beads of moisture may also occur on the leaves. As control, set up a jar with a plant stripped of its leaves.

J 3b. Take three leaves from a plant. Vaseline the upper surface of one and the under surface of another. In each case vaseline the petiole, especially at the cut surface. Leave all three in a dry place for some time. Compare the extent of shrivelling with the relative numbers of stomata on upper and lower surfaces of another leaf (determined by microscopic counts of epidermal strips; or roughly by closing the petiole with vaseline and plunging into hot water, and observing the location of the bubbles produced). (Evergreens are useful.)

J 3c. Experiment J3b can be made quantitative by weighing the leaves at intervals, using several pairs of leaves to facilitate accurate weighing and give mean results.

J 3d. Measure the absorption rates of two similar cut shoots by means of potometers. Repeat with the upper surfaces of the leaves of one, and the lower surfaces of the leaves of the other vaselined. Compare the relative effects of the two vaselinings with the relative numbers of stomata on the two surfaces of specimen leaves, removed before measurements begin. (Shoots of *Aucuba* work well.)

J 3e. Place a piece of dry blue cobalt chloride paper on the lower surface of a leaf, cover with a dry glass slide, and hold in place with a clip. The paper changes colour to pink, owing to the action of moisture evaporating from the leaf. As a control, clip paper and slide to a dry block of wood.

J 3f. Experiment J 3e can be made quantitative by timing with a stopwatch the number of seconds required for the colour to change. Whence compare the rates of evaporation from upper and lower surfaces of leaf, in sun and in shade, in

warmth and in cold, in shelter and in wind (e.g. electric fan) etc.

J 3g. Cut a shoot of a woody plant (e.g. cherry laurel) with 20–30 leaves. Vaseline the surface of the woody part (but not the cut end) of the stump, and attach by means of pressure tubing to a 2′ glass tube filled with water, making sure that there are no air bubbles in the tube. Place the tube vertically with its free end in mercury, and clamp in position. As water is absorbed, mercury passes up the tube, demonstrating the powerful force of transpiration.

J 3h. Repeat experiment J 3g, using instead of the shoot a porous pot about 3″–4″ long, joined to the glass tube by a rubber bung, the pot and tube being filled with water. This demonstrates the non-vital raising of water by the energy of evaporation.

J 3j. Wrap a nasturtium (*Tropaeolum*)leaf, still attached to the plant, around a sensitive thermometer bulb, and do likewise with a detached leaf which has been allowed to wither. The transpiring leaf registers a lower temperature owing to the cooling effect of the evaporation.

J 3k. Place two freshly cut shoots of white deadnettle (*Lamium album*) in a solution of eosin (or red ink). From one shoot remove the leaves. After ½–1 hour cut sections of the stems to see how far the solution has risen. It rises higher in the shoot with leaves.

J 3l. Pass the petiole of a leaf (e.g. cherry laurel or *Aucuba*) through a bung hole, and place the bung in the lower end of a vertical hard glass tube about 5″ long. Pour water into the tube to a depth of about 2″, covering the cut end of the petiole. In the top end of the tube insert a bung carrying a tube leading to a vacuum pump. Make all joints air-tight with plasticene, and reduce pressure in tube. Bubbles of air rise from the cut end of the petiole, indicating the presence of pores and passages in the leaf.

See also experiments F 5a–g, k.

J 4. Channel of Transpiration.

J 4a. Ring a woody shoot such as lilac or laurel (i.e. remove an annular strip of bark, about 1″ in height, and vaseline the exposed wood to prevent the entry of air). Place with the cut end in water. Similarly set up two untreated shoots, one in water, one in a dry container. The last shoot withers, the first two do not. This indicates that the bark is not necessary to the rise of water in the plant.

J 4b. Prepare two discs of wood, about ½″ diameter and ¼″ thickness, the one with its vessels running through the disc and the other with its vessels along the disc. Fix them successively with pressure tubing or a screw nozzle to the end of a tap, and turn the water on to the same extent in each case. The water comes through much more readily in the first case, indicating that wood vessels provide a good passage for water.

J 4c. Stand a plant (e.g. white deadnettle, *Lamium album*) in diluted red ink or eosin solution. After an hour cut sections of the stem at different levels. Red coloration of xylem elements can be seen. Microscopic examination shows greater detail. If the plant is left longer, the red can be seen passing along veins in the white petals.

J 4d. Repeat experiment J 4c, using a plant such as balsam, with translucent tissues. The red coloration can be observed without cutting sections.

J 4e. Repeat experiment J 4c, using a compact white cabbage. This shows up the channels exceptionally well, when cut vertically into four segments. For individual use in class, the brussels sprout serves well.

J 5. Translocation.

J 5a. Repeat experiment M 2f with artichoke leaves, but collecting the first halves in the evening and the second halves early next morning. The loss of weight is due to translocation and respiration.

J 5b. Dig up a potato plant in midsummer. Test sections of the tubers and of the intervening rhizomes for starch and sugar. The tubers contain starch, and the rhizomes sugar, in which form the carbohydrate is translocated.

J 5c. Crush separately in a mortar 50 barley grains which have soaked for 1–2 hours, and 50 with seedling leaves 1–2″ long. In each case extract with water, concentrate extract, and test for sugars. The germinated grains contain the more, owing to the hydrolysis of stored starch, prior to translocation.

J 5d. Expose a plant to light for a day and then remove one or two leaves. Test both the removed and some attached leaves for starch next morning. The latter will have lost their starch, which has been converted to sugar and translocated away.

J 5e. Place a woody plant such as lilac in the light for some hours, so that a specimen leaf gives a positive reaction to the test for starch. Now ring the stem between the leaves. De-starch in a dark cupboard and test the leaves with iodine. Leaves from above the ring give a positive result, indicating that they have not lost starch. The lower leaves gives a negative result. As a control, use an unringed plant. The experiment is explained by the fact that photosynthesised sugars (the form in which carbohydrates are transported) pass down the stem in the bark (in the phloem).

J 5f. Ring below the leaves a cut shoot of willow about 2′ long. Place the cut end in water (covering the ring with vaseline to prevent drying) together with an unringed shoot. After 2–3 weeks the latter develops roots, but the former fails to do so, since ringing has prevented the down-passage in the phloem of food from the leaves.

See also experiments B 2q–s; B 3k.

TOPIC K.

RESPIRATION

One aspect of respiration is the liberation of part of the energy of large molecules. The energy may appear as heat, muscular movement, electrical change, etc. and may be liberated either by the fragmentation of molecules (i.e. fermentation or anaerobic respiration) or by their oxidation (aerobic respiration). Fermentation is the simpler process, but liberates much less energy. It is however, doubtful to what extent this liberation of energy benefits the organism. At any rate in some plants, it has been shown that virtually all of it is dissipated as heat.

It is impossible to draw a sharp line of division between "anaerobic" and "aerobic" respiration, for the early stages of the two processes are similar if not identical.

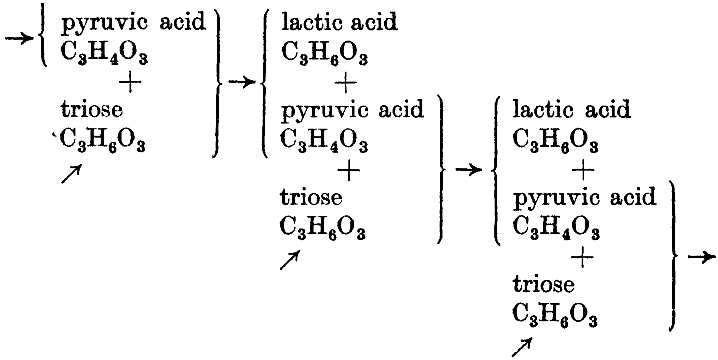
The first stage consists of the breakdown of glycogen to hexose, and then of the hexose into triose, thus:—

$$\underset{(C_6H_{12}O_6)x}{\text{glycogen}} \longrightarrow \underset{xC_6H_{12}O_6}{\text{hexose}}$$

$$\underset{C_6H_{12}O_6}{\text{hexose}} \longrightarrow \underset{2C_3H_6O_3}{\text{triose}}$$

The fate of the triose depends upon the presence or absence of oxygen, and upon the particular organism and tissue concerned.

In mammalian muscle tissue, the triose is converted into lactic acid, which has the same empirical formula, but a different constitution. This however, is not a simple process. Actually the triose loses two atoms of hydrogen to pyruvic acid ($C_3H_4O_3$) which is thereby reduced to lactic acid. The oxidation-product of the triose is pyruvic acid, which is therefore available for oxidising more triose, and so on cyclically, thus:—



In other cases, the products of fermentation may be not lactic acid, but alcohol, etc. In all cases some energy is liberated in fermentation.

In the muscle, one-fifth of the lactic acid is oxidised to carbon dioxide and water:—

$$\underset{C_3H_6O_3}{\text{lactic acid}} + \underset{3O_2}{\text{oxygen}} \longrightarrow \underset{3CO_2}{\text{carbon dioxide}} + \underset{3H_2O}{\text{water}}$$

Part of the energy liberated in this reaction is used in re-synthesising the other four-fifths of lactic acid into hexose, the remainder being available in other directions.

Thus the liberation of energy in the muscle involves the accumulation by fermentation or "anaerobic" respiration of lactic acid, which is later removed by oxidation or "aerobic respiration". Apart from this energy obtained rather readily by means of the production of lactic acid and the running-up of a temporary "oxygen overdraft", there is an even more readily available "loose cash" supply in the form of phosphagen, which breaks down to creatine and phosphoric acid with the liberation of energy. Later, the phosphagen is re-synthesised.

The whole process of anaerobic respiration in yeast is usually represented by the simple equation:—

$$C_6H_{12}O_6 \longrightarrow 2C_2H_6O + 2CO_2 + \text{energy}$$

while that of aerobic respiration is given by:—

$$C_6H_{12}O_6+6O_2 \longrightarrow 6CO_2+6H_2O+\text{energy}$$

These reactions are in reality very complex, involving the participation of enzyme and other catalytic systems. For example, at least ten enzymes are involved in the change of glycogen to lactic acid.

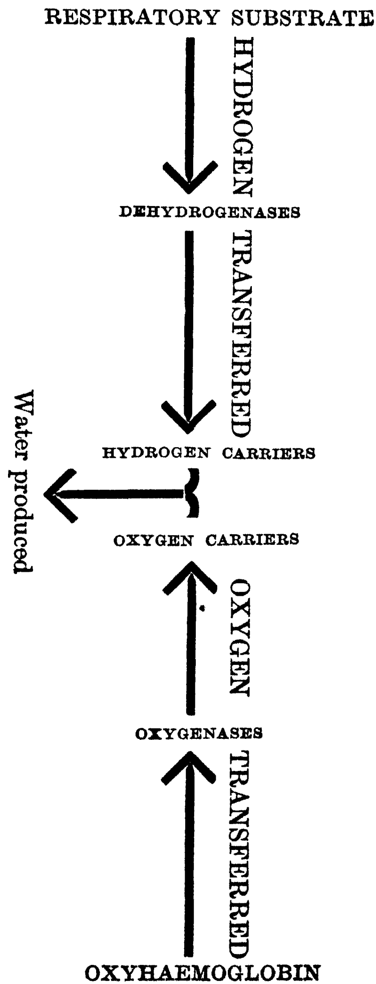
In most plants the end-products of respiration are carbon dioxide and water, but many succulents produce organic acids, Bacteria produce various monobasic organic acids, while fungi tend to produce dibasic acids such as oxalic, fumaric and citric.

In yeast fermentation, in addition to the main products alcohol and carbon dioxide, there are formed also small quantities of other substances such as acetaldehyde, glycerol, pyruvic acid and higher alcohols. The proportions produced depend upon the conditions of action and the particular strain of yeast.

Phosphates play a role of great importance in respiration, although for the sake of simplicity they are omitted from the equations given here. Thus it is not hexose, but hexose phosphates, and not triose, but triose phosphates which actually react, and the same complication applies at many stages of the process.

The molecules which are oxidised in respiration, do not combine with oxygen, but lose hydrogen. This process is aided by a whole group of enzymes called "dehydrogenases". The hydrogen is then passed on along a chain of "hydrogen carriers" —compounds which are alternately reduced (by accepting hydrogen) and then oxidised (by donating hydrogen to the next carrier). Meanwhile the oxygen, carried to the tissues by the blood, is handed on by "oxygenases" and "oxygen carriers". At one point of the complex set of chemical reactions, the oxygen and hydrogen meet and form water. It is known that in some tissues very important hydrogen carriers are the "C_4" dicarboxylic acids. Thus oxaloacetic and malic acids form one oxidation—reduction cycle, and fumaric and succinic acids another. It is also known that lactoflavin and nicotinic amide (which are constituents of vitamin B_2) play an important part

in hydrogen transfer. Among the oxygen carriers an important group is the cytochromes, which are similar in constitution to haemoglobin. The process may be represented as follows:—



The rate of respiration is affected by external factors such as oxygen and carbon dioxide concentrations of the air, temperature, and so on. It is also affected by internal factors such as the concentration of respirable materials in the cell, the cell's water content, and the type, age and previous history of the cells. Although carbohydrates are of primary importance as substrates for respiration, it should be remembered that fats and proteins may also be respired.

The method of gas exchange in plants is fairly simple, depending in most cases upon diffusion through the stomata. Animals display many interesting methods of gas exchange. The smallest (e.g. *Amoeba,* small crustaceans) depend on diffusion through the surface, while other aquatic organisms depend on gills. This is true of the tadpole (first external, later internal); fish (water passed over the gills by the muscular movement of the floor of the mouth); crayfish (water enters the gill chamber near the base of the legs, and leaves below the antennae, being circulated by the movement of appendages); many early stages of aquatic insects; the mussel (water passed over the gills and mantle by the action of cilia); and so on. Insects have an almost unique mechanism, in their tracheae (air tubes) opening to the exterior by spiracles. Most land animals have lungs (bag-like as in reptiles, alveolar as in mammals, or with air sacs as in birds). Mammals cause the air to enter and leave their lungs by movements of the diaphragm and ribs. The frog is of special interest, gas exchange occurring in lungs, in the mouth, and through the moist surface of the body.

The rate of breathing in many animals (e.g. humans) is regulated by the carbon dioxide in the blood, being rapid with high concentration, and slow with low.

Experiments are classified as follows:—

K1. Absorption of Oxygen.
K2. Production of Carbon Dioxide.
K3. Liberation of Energy.
K4. Anaerobic Respiration.
K5. Mechanism of Gas Exchange.

See also experiments E 1d; M 2d, e.

K 1. Absorption of Oxygen.

K 1a. Hold the nose and breathe the same air through glass and rubber tubing out of and into a gas jar (containing air) inverted over water. Insert a lighted splint. It is extinguished, indicating that oxygen has been absorbed from the air during breathing.

K 1b. Cover the bottom of a gallon winchester bottle with soaked peas, and insert a tightly fitting bung. Leave for about a week, and then remove the bung. Insert a lighted splint into the bottle. It is extinguished, owing to the absorption of oxygen by the respiring peas. As a control set up a parallel set of apparatus without peas.

K 1c. Suspend a plant root in one arm of a U-tube (preferably with wide necks but a narrow bend) by means of a thread held in place by the bung. Pour limewater into the bottom of the tube, and insert a bung into the other opening. Leave for some days, when the limewater turns milky and rises in the arm containing the root, owing to the absorption of oxygen during respiration, and the production of carbon dioxide which is then absorbed by the limewater. Set up a control experiment, using oil (which does not absorb carbon dioxide) instead of limewater. (Limewater may be replaced by potash).

K 2. Production of Carbon Dioxide.

K 2a. Place limewater in two flasks having two-holed bungs, each with one short glass tube and one long one dipping into the lime water. Join a T-piece by means of rubber tubing to the long tube of one flask and the short tube of the other. Breathe in and out through the T-piece, so that air bubbles through one volume of limewater and out through the other. The latter turns milky first, indicating that respiring humans produce carbon dioxide.

b. Take two similar bowls of water. Place in one some

goldfish or sticklebacks and leave for a day. Then add a sample of water from each bowl to some limewater. The first sample causes milkiness, indicating that respiring fish produce carbon dioxide.

K 2c. Place some limewater to the depth of 1″ in a gas jar. In the jar suspend some soaked peas in a muslin bag, and cover with a vaselined glass lid. The limewater turns milky in time, indicating that respiring peas produce carbon dioxide. The experiment may also be carried out with earthworms, leaves, fruits, tubers, etc., kept in the dark. Carry out a control experiment using an empty muslin bag.

K 2d. Place some damp loamy soil in a muslin bag, and suspend in a gas jar containing limewater. Cover with a vaselined glass lid, and leave in a warm dark place for a couple of days. The limewater turns milky, owing to the evolution of carbon dioxide in the respiration of soil micro-organisms. Carry out control experiments with well baked soil in a muslin bag, and with an empty muslin bag.

K 2e. Place roots of some healthy wheat seedlings in a jar of water tinged with faintly alkaline cresol red. The dye changes colour owing to the evolution of carbon dioxide. Carry out a control experiment without any roots in the liquid.

K 2f. Add 6–8 drops of an indicator (e.g. 0·04% cresol red) to 10 ml. water in a boiling tube. Place in the solution a small aquatic animal, cork and leave for 5–10 minutes. A change of coloration indicates increased acidity, due to the production of carbon dioxide. Carry out a control experiment without any animal in the liquid.

K 2g. Place a potted plant in a bell jar standing on a vaselined glass sheet. In the neck of the bell jar place a two-holed bung, with entry and exit glass tubes. By means of an aspirator draw air through the following in succession—i. A flask of potash solution to remove carbon dioxide. ii. The bell jar. iii. A flask of limewater. Keep the bell jar in the dark

prevent photosynthesis (this can be done by means of a heavy black cloth). The limewater goes milky, indicating the production of carbon dioxide by the respiring plant. As a control, set up a parallel set of apparatus containing pot and soil but no plant, thus correcting for any action by soil.

K 2h. Place a measured volume of very dilute sodium carbonate solution in a boiling tube (a suitable dilution can be found from one or two preliminary experiments). Colour faintly with a drop or two of phenolphthalein solution. Drop in six soaked peas. As they respire, the carbon dioxide evolved converts the carbonate to acid bicarbonate and turns the phenolphthalein colourless. Record the time taken, together with the temperature of the liquid. Now repeat the experiment, placing the tube in a warm water bath, with a fresh equal volume of carbonate solution but the same peas. Repeat at various temperatures and plot graphically the temperature against the reciprocal of the time taken.

K 3. Liberation of Energy.

K 3a. Place a sensitive thermometer in each of two vacuum flasks. In one place a handful of soaked peas and in the other an equal number of peas killed by boiling. Plug the necks of the flasks with cotton wool. Read the two thermometers at intervals, recording the results. The first shows a significant rise in temperature, indicating that peas produce heat in respiration. The dead peas may possibly show a rise in temperature, owing to growth and respiration of micro-organisms; and a further rise may occur with the previously living peas after their temperature rises so high that they are killed. Such eventualities present valuable opportunities for discussion, but the former may be prevented by moistening the dead peas with a solution of formalin. The thermometers should be compared at the start of the experiment.

K 3b. Repeat experiment K 3a using earthworms on wet otton wool in the first flask, and wet cotton wool only in the cond. The temperature rise indicates that earthworms pro-

duce heat in respiration. Remove the earthworms as soon as the rise has been observed, to avoid ill-effects of heat.

K 3c. Place several bumble bees (or bluebottles) in a small vacuum flask, and record the temperature by means of a thermometer inserted in the neck. It may rise as much as 3° C.

K 4. Anaerobic Respiration.

K 4a. Completely fill a test tube with mercury, and invert in mercury in a trough. Pass six soaked peas into the tube. They rise to the top. In a day or two the mercury is forced down the tube owing to the production of a gas. After the mercury level has fallen, introduce with a pipette a drop or two of limewater into the tube. It rises to the top, and absorbs the gas, the mercury thereupon rising again. Milkiness of limewater indicates that the gas contains carbon dioxide. Carry out control experiment with baked (killed) peas. The seed coats should be carefully removed from the peas before they are introduced into the tube, to ensure that no air is carried within them.

K 4b. Cover the bottom of a strong bottle (about ½ pint) with soaked peas. Insert a well-fitting bung and leave for about a week. Hold the bottle with the cork between two forefingers, and gradually loose the cork. When it is sufficiently loosened, it is projected with fair velocity owing to the high gas pressure within the bottle. (Carbon dioxide accumulates by anaerobic respiration after the oxygen is used up, and other gases are produced in decomposition.)

K 4c. Place a little yeast in 10% glucose solution in a boiling tube, and cover the tube with a rubber balloon. Set up a control experiment without yeast. Place both tubes in a water-bath at about 30° C. and leave. The balloon on the yeast-containing tube is inflated with the gas (carbon dioxide) evolved.

K 4d. Place 500 ml. of a 10% solution of glucose, well mixed with 15 gm. of brewers' yeast, in a very large flask. Fit the

flask with a rubber bung, through which passes a glass tube dipping into limewater. Keep the apparatus in a warm place. The mixture soon froths, and the limewater is turned milky owing to the evolution of carbon dioxide. There is also a quite marked smell of alcohol, which may be distilled off after a couple of days from the pump-filtered mixture (using a Liebig condenser). After redistillation, the first fraction will probably burn quite readily. A control apparatus should be set up without any yeast.

See also experiments D 1a, b.

K 5. Mechanism of Gas Exchange.

K 5a. Breathe out, close the mouth, pinch the nose and expand the chest as much as possible. Now release the nose, when air is "sucked in", owing to the reduction of air pressure in the lungs by chest expansion.

K 5b. The class splits into pairs. Each person measures the partner's chest expansion, the results being recorded communally. The vital lung capacity is measured by breathing out fully through a tube into a bell jar standing in water, after first taking several deep breaths. The bell jar must have a removable stopper, so that the water level may be restored after each measurement. Down the side of the bell jar is a graduated scale, along which the volume of water displaced may be read in arbitrary units. The results show that there is no simple correlation between chest expansion and vital lung capacity. By breathing out normally, the tidal air volume may also be measured.

K 5c. Breathe the same air into and out of a football bladder several times, and observe how the increasing carbon dioxide concentration in the blood causes an increase in the frequency of breathing. Potash solution in the bladder prevents this, since it absorbs the carbon dioxide produced.

K 5d. Breathe in and out deeply and quickly for as long as possible. The decreasing carbon dioxide concentration in the

blood causes slowing of breathing and eventually makes it almost impossible to breathe. Carbon dioxide then accumulates, and breathing begins again.

K 5e. Count the number of breaths per minute for as many people as possible of different ages, ranging from young babies to adults. Calculate the mean rate for each age (taking "age last birthday" so that young babies will be of age 0). Plot mean-rate against age-in-years. The graph shows a marked reduction in rate with increasing age, especially during the first few years.

K 5f. Mount in water part of the gill of a fresh water mussel, and sprinkle on it fine metal filings. Observe microscopically the movement of the filings by the cilia.

K 5g. Observe a living frog, and note the periodic forcible "lung breathing" as well as the regular "mouth breathing".

K 5h. Observe the movements of the mouth and branchiostegal membrane of a living goldfish.

K 5j. Just cover a living crayfish with water, and by means of a small pipette run a carmine-water suspension below the thorax between the 2nd and 3rd walking legs. A coloured stream appears in front of the head, where the "respiratory current" leaves the animal.

K 5k. Similarly demonstrate by means of carmine, the currents of water into and out of the rectum of a dragonfly larva in a watchglass.

See also experiments A 2e–g; A 6h; H 2e; H 4b–f; L 2a.

N.B.—For gas exchange in higher plants see experiments on Stomata, F 5a–k; J 3b–f.

TOPIC L.

EXCRETION AND VOIDING

The word "excretion" should be used precisely. It is the separation of the waste by-products of the chemical activities of the cells by the activity of the tissue concerned. The passing of the excreted material from the body is known as "voiding". Thus the kidney tubules excrete urea, while the bladder voids urine. So-called "excreta", consisting largely of unabsorbed food, bacterial cadaver, etc. contain very little excreted material. Their voiding is called "defaecation".

The chief excretory organs vary greatly from one animal group to another. *Hydra* for example, has no special organs, the waste materials merely diffusing out of the body cells. The tapeworm has "flame cells" which pass excreted material into canals; while the earthworm has excretory tubes (nephridia) in most of its segments. The snail has one excretory tube starting near its heart; the crayfish two "green glands" in its head, opening near the bases of the antennae; while insects have anything up to about a hundred excretory tubes opening into the alimentary canal. Vertebrates have the excretory tubules elaborated into two definite kidneys of great complexity.

The material excreted from the kidneys contains a good deal of urea in fish, amphibia, and mammals; while birds and reptiles produce considerable uric acid. Other nitrogenous materials excreted in varying proportions are ammonia, creatinine, hippuric acid, etc.

Excretion in plants takes a variety of forms. Where crystals (e.g. calcium oxalate) are formed by the tissues as a by-product of metabolic activity, they may be properly regarded as excreted material. Other excretory products of plants are resins, tannins, ethereal and essential oils, and some alkaloids and pigments. Plants differ from the higher animals in usually not possessing such definite excretory organs, and in retaining the excreted material instead of voiding it. (Although leaf-fall may be regarded as a form of voiding.)

Very few simple experiments may be carried out on excretion and voiding, but various excretory products (both plant and animal) should be demonstrated.

Experiments are classified as follows:—

L1. General.
L2. By the Lung.
L3. By the Skin.
L4. By the Kidney.

L 1. General.

L 1a. Observe a living *Amoeba* mounted in pond water on a slide. Excretory products diffuse out over the surface. Note that after the ingestion of food particles, solid material may be voided from the animal.

L 1b. Repeat experiment L 1a with living *Paramecium.*

L 1c. Place a freshly killed earthworm in normal saline, and remove a nephridium. Mount in the same liquid on a slide and observe microscopically. Note the beating of the cilia which cause a current of excretory liquid to flow through the tube. Note also the network of fine blood vessels from which the nephridium extracts waste materials in solution.

L 2. By the Lung.

L 2a. Breathe out several times through a glass tube dipping into a beaker of limewater. The limewater turns milky, indicating that the expired air contains carbon dioxide.

L 2b. Breathe out onto a cold polished mirror. The surface becomes clouded, indicating that the expired air contains water vapour.

L 2c. Breathe out into a tube dipping into diluted Jeyes' fluid (or potassium permanganate solution). Any organic matter in the breath turns the fluid brownish.

L 3. By the Skin.

L 3a. Examine the skin of the hand microscopically by means of reflected light. Pores (the openings of sweat glands) can be seen, especially on the palm.

L 3b. Wear a rubber finger-stall for about $\frac{1}{2}$ hour, and thus accumulate sweat in it. Place in the sweat a clean platinum wire, and then place in a bunsen flame. A brilliant yellow coloration indicates sodium in the sweat.

L 3c. Wear a rubber finger-stall for about $\frac{1}{2}$ hour, and then rinse out with diluted Jeyes' fluid (or potassium permanganate), pouring back into a test-tube for observation. A coloration indicates organic material in the sweat.

L 3d. From some dead cockroaches scrape off the outer surface, and place the scrapings in a hard glass ignition tube. Mix with soda lime and heat. Ammonia is evolved, and can be detected by its smell and action on damp red litmus paper. The nitrogenous layer which accumulates outside the insect is excreted material.

L 3e. Similarly test for nitrogen the chitin obtained by leaving a crayfish skeleton for a few days in dilute hydrochloric acid, washing, boiling for $\frac{1}{2}$–1 hour in dilute sodium hydroxide, and re-washing.

See also experiment B 31.

L 4. By the Kidney.

L 4a. Heat some urine gently in an evaporating basin, until most of the water has evaporated. Then allow it to crystallise at room temperature. Extract with acetone, re-crystallise, and place a few crystals on a microscope slide. Examine microscopically and compare with a slide of urea crystals. The similarity of crystalline form indicates that the urine contains urea.

L 4b. Evaporate some urine to $\frac{1}{4}$ of its bulk, and add a few drops of concentrated nitric acid. Examine microscopically. Crystals of urea nitrate can be seen.

L 4c. Add a little concentrated hydrochloric acid to urine in a test tube, and allow it to stand for a day. A pinkish precipitate of uric acid slowly forms and sediments.

L 4d. Moisten a little bird excrement on a slide with nitric acid, and warm until yellowish-red. Add a drop of ammonia solution. A purple colour (the murexide test) indicates that bird excrement contains some uric acid.

L 4e. To about 3 ml. urine in a test tube add a few drops of concentrated nitric acid and about 3 ml. dilute silver nitrate solution. A white precipitate of silver chloride indicates the presence of chloride in the urine.

L 4f. To about 3 ml. urine in a test tube add a few drops of concentrated hydrochloric acid and 2–3 ml. barium chloride solution. A white precipitate or opalescence of barium sulphate indicates the presence of sulphate in the urine.

L 4g. To urine in a test tube add a little concentrated ammonia solution and boil. A white precipitate is formed, containing calcium phosphate. Filter off the precipitate and dissolve in dilute acetic acid, dividing the solution into two parts. To one part add potassium oxalate solution. A white precipitate of calcium oxalate indicates calcium. To the other part add concentrated nitric acid and ammonium molybdate solution and boil. A yellow precipitate indicates phosphate.

See also experiment G 4g.

TOPIC M.

GROWTH

Growth cannot be defined by any single measure. It is a process involving changes in dimensions, weight, form, etc. and depending upon many features of the environment, as well as on the inherent growth tendency of the organism. It is important to carry out sufficiently varied experiments to make clear this multiformity of growth.

Growth is usually considered as the addition of living material, and is represented graphically by plotting a measure (m) against time (t). The additive growth rate at any moment $=dm/dt$, which is the gradient of the curve. It is however, often instructive to consider growth as a process of multiplication of living material. In this case, the multiplicative growth rate at any moment$=dm/mdt=d$ (log m) $/dt$, which is the gradient of the plot of log m against t.

Let k_1 be the multiplicative growth rate of one part of an organism at any moment, and k_2 that of another part or of the whole. Then $k_1=d$ (log m_1) $/dt$ and $k_2=d$ (log m_2) $/dt$. Therefore $k_1/k_2=d$ (log m_1) $/d$ (log m_2), which is the gradient of the plot of log m_1 against log m_2. Often this plot is a straight line, the ratio k_1/k_2 remaining constant. If $k_1=k_2$, growth is said to be "isometric", and the gradient is 45°. If k_1 is not $=k_2$, growth is "allometric", and the gradient is $>$ or $<$ than 45° according as k_1 is $>$ or $< k_2$.

Allometric growth is responsible for change of bodily form, and this change is indicated by any deviation from a straight line, of the plot of the cube of a linear dimension, such as length, against volume or (approximately) weight.

Growth rate is affected by temperature, being accelerated up to a certain critical value, and retarded above it. Light affects growth in many ways, especially in plants. Apart from its obvious effect on photosynthesis, light has far-reaching but little understood formative influences, affecting tuber formation, etiolation, hydrogen ion concentration of cell sap, type

of root development, and degree of reproductive activity.

Food also has important effects on growth, and in plants the relative amounts of carbohydrates and of nitrogenous compounds have an influence upon the type of growth. A serious lack either of carbohydrates or of nitrogenous compounds leads to weak vegetative growth and sterility, high values of both often lead to vigorous vegetative growth with sterility, while an abundance of carbohydrate with comparatively little nitrogen often gives rather weaker vegetative growth with fertility.

Growth in higher plants and animals is largely regulated by the action of hormones, chemicals of extraordinary potency.

Experiments are classified as follows:—

M1. Change in Dimensions.
M2. Change in Weight.
M3. Change in Form.
M4. Effect of Environment.
M5. Effect of Food.
M6. Effect of Hormones.

See also experiments Q 1a–e.

M 1. Change in Dimensions.

M 1a. Attach a thread to the tip of a potted plant, pass it over a pulley, and at its other end tie a small weight. Behind the weight place a graduated vertical scale. Record the position of the weight on the scale daily. Plot the results graphically, showing growth in length with time.

M 1b. Experiment M 1a may be modified to magnify the growth readings, by using a light lever attached by its shorter limb to the top of the plant, and with the tip of its longer limb passing over a vertical scale.

M 1c. Experiment M 1b may be modified, placing a metal sheet with a pin hole in front of the plant, and a vertical scale 10–15′ away, thus using line of vision as a "magnifying lever" which is weightless. Move the eye up and down by the scale until the tip of the plant is seen through the pinhole, and

record the scale position of the eye. The readings on the scale must be divided by the ratio of the distances of the scale and the plant from the pinhole, to obtain absolute values.

M 1d. Similarly record and graph the growth in height of a selected dandelion or coltsfoot in the open or in a pot, beginning the record as soon as the plant appears above ground.

M 1e. Obtain a similar curve for humans, using official statistics of heights, or deriving them from pupils, staff, etc.

M 1f. With a sharp needle make a scratch on the left thumb nail near to the base and parallel to it. Each week measure very carefully in millimetres the distance from the scratch to the base, and represent the results graphically.

M 1g. Leave some planarians in tap water without food, and measure their size (e.g. length and width) once each week. A negative growth of size occurs and may go on for months.

M 2. Change in Weight.

M 2a. A fortnight after the birth of a pet mouse, weigh it in a weighed box. Repeat the weighing daily. Record the results in a table, and plot weight against age graphically. A sigmoid curve is obtained with a central steep part, representing the "grand period of growth".

M 2b. Obtain a similar curve for humans, using official statistics of weight, or deriving them from pupils and staff.

M 2c. All the members of the class weigh themselves weekly on the same machine, and always at a comparable time, e.g. after morning school. Plot graphically the increase in weight with time.

M 2d. Plant 200 soaked peas of a pure strain in a box of loose damp sawdust. Water uniformly at intervals. Immediately, and at intervals of 5 days, remove 20 peas at random, taking care not to disturb unduly those remaining. Wash off loose sawdust, and chop up in a weighed vessel. Heat in a water

bath, cool, and weigh. Repeat until the weight is constant. Whence obtain a table and graph showing growth of peas as indicated by dry weight. (Note initial reduction of weight, due to respiration.)

M 2e. Take a potato in the spring, and allow it to grow without giving it any water. Weigh at intervals, and demonstrate growth with loss of weight due to respiration.

M 2f. With an inked rubber stamp mark out equal areas (say 50 sq. cm.) on each side of the midveins of 100 leaves of Jerusalem artichoke. Early in the morning cut out the marked areas from one half of each leaf and dry the collection in a steam oven until its weight is constant. In the early evening repeat with the other halves of the leaves. The growth in weight due to photosynthesis may then be calculated in gm. per sq. cm. of leaf area. The result is not accurate since no allowance is made for the effects of respiration.

See also experiment E 1d.

M 3. Change in Form.

M 3a. Place three seeds on a long tray of wet sawdust each day over a period. Keep the sawdust moist. After the last set of seeds has germinated, the development of the seedling is visible in the series, showing change in form.

M 3b. Place hens' eggs in an incubator, one each day, numbering them successively. When the first egg shows signs of hatching, break open the eggs carefully and empty the contents into numbered dishes containing enough normal salt solution to cover their bottoms. Observe the development of the chick embryo, as shown in the series.

M 3c. At intervals of a week, trace on to squared paper the outline of a runner bean leaf, over as long a period of its development as possible. Now reduce the tracings to a common length on a single sheet of graph paper, and observe change of form during growth.

M 3d. Obtain a series of photographs of an animal (e.g. frog, cat, horse, human), at intervals of its life. Trace their outlines, and on graph paper reduce each tracing to a common length. The change of form during growth is very obvious.

M 3e. Mark the radicle of a broad bean seedling at intervals of 1 mm. by means of cotton dipped in indian ink and held taut. Leave pinned against cork with the radicle pointing earthwards in a gas jar kept moist with wet cotton wool. The movement of the ink marks indicates that growth is most rapid a little way behind the tip.

M 3f. Mark the stem of a 3″ bean seedling at intervals of ¼″ by means of cotton dipped in indian ink and held taut. Fix upright with roots in water, and examine daily. The movement of the ink marks indicates that growth is mainly towards the tip, but is much more diffused than in the case of a root.

M 3g. Observe the development, moult by moult, of the brackish water shrimp (*Gammarus chevreuxi*). The growth of the secondary male characters is particularly interesting.

M 3h. Make measurements of abdomen breadth (l_1) and carapace length (l_2) of a large number of female crabs (e.g. *Carcinus maenas*), covering a wide range of size. Plot log l_1 against log l_2. The gradient of the line indicates positive allometry of l_1. Repeat with male crabs, which show approximate isometry.

M 3j. Repeat experiment M 3h, measuring lamina diameter (l_1) and petiole length (l_2) of a large number of *Tropaeolum* leaves. There is positive allometry of l_1.

M 3k. Repeat experiment M 3h, measuring tail length (l_1) and trunk length (l_2) of a mouse throughout as much of its life as possible. There is positive allometry of l_1.

M 3l. Adapt experiment M 2a, measuring trunk length (l) as well as weight (w) of the mouse. Plot w against l^3. Any deviation from a straight line indicates a change of bodily form during growth.

M 3m. Combining the figures of experiments M 1e and M 2b, plot l^3 against w. Any deviation from a straight line indicates a change of bodily form during human growth.

See also experiments E 1a, b.

M 4. Effect of Environment.

M 4a. Soak 6 broad beans and plant in small pots. Keep 3 pots in a warm place, 3 in a cold. Record for each plant the date of appearance of each leaf. The plants which have been kept warm develop the more rapidly.

M 4b. Place equal quantities of frog spawn from the same mass into two jars of water, and keep one in a warm and the other in a cold place. Compare their development daily.

M 4c. Experiment M 4b may be made quantitative, by keeping several batches of frog spawn at known different temperatures, and recording the time taken to reach certain arbitrary stages (e.g. appearance of external gills, rear legs, front legs; loss of tail, metamorphosis).

M 4d. Prepare two similar petri dish cultures of bacteria or fungi, and compare development in warmth and in cold.

M 4e. Record the dates of flowering of various species on the two sides of a lane with northerly and southerly aspects respectively, over a period of weeks. Also record the soil temperature of the two sides of the lane daily over the same period. The difference is often quite marked.

N.B.—This does not *prove* that temperature difference is the effective agent of earlier flowering.

M 4f. Experiments M 1a–c can be modified to give hourly readings in day and at night, in warmth and in cold, in flood and in drought, etc.

M 4g. Etiolation in darkness can be well observed by allowing potato tubers to grow, some in the light and some in the dark, for several weeks.

M 4h. Germinate 6 sets of broad beans in the dark. Keep one set in the dark, one in the light, expose one to light for 1 minute, one for 2 minutes, one for 5 minutes and one for an hour each day. The seedlings give a series grading from complete etiolation to normal development.

M 4j. Place equal numbers of young tadpoles from the same mass of spawn into 6 similar glass dishes, with similar amounts of the same sort of water. Place one dish in good indirect light and one in the dark; one in a warm place and one in a cold; one on black cloth and one on white. As the tadpoles grow, keep a diary of their development and appearance. Whence determine the effects on growth of light, temperature, and colour of background.

See also experiments E 2a, c; E 3a–e; E 4a, b; E 5a–c; F 3d.

M 5. Effect of Food.

M 5a. By growing plants in solutions containing various mineral elements, the effects of absence of any of them can be observed. Germinate barley on damp sawdust for 10 days (preferably in February or early April) and transfer one seed to each solution (including distilled water and complete solution). Roots only should dip in, the seed being kept scrupulously dry (to avoid fungal infection) in a hole in a scalded and dried cork, fitting in a glass bottle of about $\frac{1}{2}$ litre capacity, filled to within 1″ of the cork. First clean the containing bottles carefully with nitric acid, tap water and distilled water. Use only distilled water in making up the solutions, and use the purest chemicals. Aerate solutions daily if possible and keep the roots of the plants in darkness. Change the solutions fortnightly, and as water is transpired, fill up with fresh distilled water. Keep the stock bottles well stoppered in the dark and shake them thoroughly before use, to ensure complete solution of the salts. Keep accurate records of length of shoot, amount of root development, number of leaves, colour of shoot, general appearance of vigour, etc. (*See* R 1h.)

M 5b. Repeat experiment M 5a, using *Tradescantia* cuttings instead of seedlings.

M 5c. Repeat experiment M 5a, using twenty healthy fronds of *Lemna* (duckweed) instead of a seedling in each solution. Over a period of about a month marked differences in growth and reproduction are noticeable.

M 5d. Wash good quality silver sand several times with dilute sulphuric acid, tap water, and finally distilled water. Place in clean new flower pots, and moisten with the various culture solutions (including complete solution and distilled water). Sow poppy seeds in the sand and observe the effects of absence of various minerals. (*N.B.*—Sand will usually retain some iron, even after the above treatment.)

M 5e. Germinate several broad beans in damp sawdust. When the plumules are just beginning to show above the sawdust, remove the cotyledons very carefully from half of them with a sharp scalpel. These seeds grow much more slowly than the untreated ones, owing to the absence of stored food.

M 5f. Place equal quantities of mustard seeds in two pots of soil, and allow to germinate. Place each pot under a bell jar. In one case accompany by a small beaker of soda lime, and fill the glass tube entering the bell jar with soda lime also. In the other case use broken stones instead of soda lime, as a control. In the first jar the seedlings receive no carbon dioxide, since it is absorbed by soda lime. It is clear after a few weeks' growth that the seedlings in this case, deprived of carbon dioxide, are not growing properly.

M 5g. Add varying small amounts of iodine to several bowls of water containing tadpoles, and observe varying rates of development.

M 5h. Place equal numbers of very young tadpoles from the same batch into two aquaria. Feed one set on young water weeds, and the other on frequently changed pieces of meat.

When fairly large, kill and dissect. The vegetarians develop a much longer gut, which helps in digesting plant material.

See also experiments F 3g, h.

M 6. Effect of Hormones.

M 6a. Place ten tadpoles from the same batch in each of two bowls of water, containing water weeds. To one bowl add part of a powdered tablet (1 grain) of thyroid extract. The tadpoles in this bowl metamorphose rapidly. This experiment however, is not as easy as it sounds. Often the metamorphosis is so rapid that the tadpoles are killed or distorted. If this happens, repeat, using less of the thyroid extract.

M 6b. Experiment M 6a may be made quantitative, by giving several equal batches of tadpoles in equal volumes of water, different amounts of thyroid extract (e.g. 1, $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{8}$, $\frac{1}{16}$, $\frac{1}{32}$, $\frac{1}{64}$ tablet), and measuring the rates of growth of limbs and of shrinkage of tail.

M 6c. Smear one side of young maize (*Zea mays*) seedlings with lanoline paste containing 1/3000 indolacetic acid (or a commercial preparation of "growth hormone"). The seedlings bend away from the smeared side, and may actually form complete loops. As a control, smear other seedlings with lanoline paste alone.

M 6d. Repeat experiment M 6c, using broad bean radicles. Note that in this case curvature is towards the side smeared, since in roots the hormone does not increase, but decreases growth in length.

M 6e. Take 20 similar cuttings of a plant, and smear the ends of 10 of them liberally with an auxin or similar preparation, marking these cuttings with a piece of coloured thread. Plant out all the cuttings, and at intervals examine their root development. The treated cuttings produce new roots more quickly than the others.

See also experiments M 5g; N 1a–c; N 2a, b, p.

TOPIC N.

SENSITIVITY

The capacity to respond to a stimulus (i.e. sensitivity or irritability) is one of the most striking characteristics of living organisms. This whole subject has been the object of a good deal of reconsideration of late, and it is probably best to keep to the plan of distinguishing between the following types of response:—

A "tropism" is a bending of part of a sessile organism in a direction determined by the direction of the stimulus.

A "taxis" is a bodily movement of a motile organism in a direction determined by the direction of the stimulus.

An "orientation" is a placing of the body of an organism in a direction determined by the direction of the stimulus.

A "kinesis" is a change of rate of movement of a motile organism in response to a change of intensity of a stimulus. (The aggregating effect of a kinesis is often superficially similar to that of a taxis).

A "nasty" is a movement of part of a sessile organism in response to a change of intensity of a stimulus, but in a direction determined by the inherent properties of the organism.

The term descriptive of the type of movement may be prefixed by a term descriptive of the type of stimulus.

e.g.	photo - - - -	(light)
	thermo - - - -	(temperature)
	geo - - - -	(gravity)
	chemo - - - -	(chemical)
	hydro - - - -	(water)
	hygro - - - -	(humidity)
	rheo - - - -	(flow)
	thigmo - - - -	(contact)
	seismo - - - -	(shock)

A response may be "positive" (towards the stimulus), "negative" (away from it), "plagio" (obliquely to its direction), or "dia" (at right angles to its direction).

The lower organisms have fairly simple receptors (e.g. the "eye spot" of *Euglena*), while in the higher animals the nervous system is involved, often in great complexity, both in reception of the stimulus and in reaction to it. In higher plants, tropisms have been shown in several cases to be due to the production in the sensitive region of hormones which travel to other parts and cause acceleration or retardation of growth, and hence curvature. Many nastic movements are effected by means of changes of turgor, due to alterations in the permeability of the cell membranes.

Experiments are classified as follows:—

N1. Phototropism.
N2. Geotropism.
N3. Other Tropisms.
N4. Phototaxis.
N5. Other Taxes.
N6. Orientations.
N7. Kineses.
N8. Nasties.
N9. Complex Reactions.

N 1. Phototropism.

N 1a. Plant soaked oat or canary grass seeds near the surface of moist soil in a box. Place the box so that light reaches it from one side only. As the seedlings grow, their coleoptiles incline towards the lighted side (positive phototropism).

N 1b. Repeat experiment N 1a, but cut off the terminal 1/10″ of the coleoptiles before exposing to light, using a red light during this operation. No inclination takes place, indicating that the tip is the region of the coleoptile sensitive to light.

N 1c. Repeat experiment N 1b, covering the coleoptile tips with caps of metal foil instead of cutting them off.

N 1d. Sow cress seeds on a tray of damp sand. Place in a box with a glass window at one end. Observe daily. The seedlings grow towards the light. Turn the tray in the opposite direction, and repeat the observations. The seedlings bend, giving a definite "S" shape (positive phototropism). Grow another tray of seeds in non-directional light as a control.

N 1e. Place a few mustard seedlings in holes in cardboard over a flat-sided glass container, their roots dipping into water. Allow light to enter container by only one side. In a few hours the roots bend away from the light (negative phototropism).

N 1f. Illuminate a potted nasturtium (*Tropaeolum*) plant from one side only. The leaves place themselves at right angles to the light direction (dia-phototropism).

N 1g. Tie a raspberry cane in the garden so that the undersides of its leaves are exposed. Eventually the leaves turn their upper surfaces to the sun (dia-phototropism).

N 1h. Grow a plant of sunflower in a garden with a southerly aspect. On a sunny day, record the compass direction of the flower-head at hourly intervals, and also the compass direction of the sun. Show graphically how the flower-head follows the sun round, keeping at right angles with its rays (dia-phototropism, or possibly thermotropism).

N 1j. Set some broad beans in a pot of soil. Stand the pot in the corner made by a cupboard with a wall, the corner being shaded by the cupboard. The stem grows diagonally out from the wall towards the light and then diagonally across the front of the cupboard (positive phototropism). Growth is rapid and an etiolated plant is produced.

N 1k. Repeat experiment N 1a on phototropic curvature, using sheets of glass of different colours. Thus determine which wave-lengths of light (mainly blue) act as stimulus.

N 2. Geotropism.

N 2a. Select a bean seedling with a radicle about 1″ long

Mark the radicle with indian ink, at intervals of about one millimetre (this can be well done by means of a thread soaked in the ink and held taut). Fix with radicle horizontal in a wide jar containing wet cotton wool dipping into water, to keep the atmosphere moist. After a day, observe the radicle. It will have bent vertically (positive geotropism), and the spacing of the marks shows that growth has been most rapid in the region originally about ½" from the tip.

N 2b. Repeat experiment N 2a with a bean seedling from which the terminal 1/10" of radicle has been cut off. No bending takes place, indicating that the tip is the region of the radicle sensitive to gravity.

N 2c. Repeat experiment N2a with alkaline pyrogallol in the jar, and an air-tight bung in its neck. There is no geotropic response, owing to the absence of oxygen.

N 2d. Fasten a young sunflower seedling with its root along a straight line ruled horizontally on a sheet of cardboard held in a vertical plane. Observe carefully, and record the time required for curvature to become apparent (positive geotropism). It usually occurs within a few minutes.

N 2e. Place two long grass stalks with prominent nodes horizontally on a flat cork. Embed the roots of each in sawdust, which should be kept really moist throughout the experiment. Pin down one stalk at each side of a node, and the other only at the side nearer the root. Leave in a dark place. In about a week the latter stalk will once more be vertical above the node, which has grown particularly on the lower side (negative geotropism).

N 2f. Place a cut flower of *Narcissus* with its stalk dipping into a little water in a test tube, and tilt the tube in a stand through an angle of about 45°. The corolla tube adjusts itself so that it is once more horizontal (dia-geotropism).

N 2g. Germinate a seed which produces clear secondary roots, in a box of soil close against a glass side, so that the roots are

visible. Keep the glass covered during germination. Now tilt the box through an angle of about 45°, and observe that after a few days the secondary roots have grown so that they make the original angle with the vertical (plagio-geotropism).

N 2h. Observe the seasonal growth of the wild field poppy. The buds are pendant, the flowers facing sideways, and the fruits erect. This means that the flower stalk is first positively, then transversely (dia-) and finally negatively geotropic. Similarly bluebells are first erect, then pendant, then erect.

N 2j. Fix a bean seedling with its radicle resting horizontally on the surface of a dish of mercury (covered by a thin film of water to keep the bean moist). Leave in a dark place to exclude any light-effect. After a day, the radicle is seen to have bent downwards against the resistance of the mercury (positive geotropism). As a control, set up a parallel experiment with a bean seedling which has been killed by chloroform.

N 2k. Place a grass stalk inside a glass tube which it fits fairly closely, and place horizontally on a bench. The grass cannot curve into the vertical, and the pulvinus in consequence becomes swollen and distorted.

N 2l. Set up some bean seedlings on the edge of a clinostat, rotating in a vertical plane to equalise any gravitational effect. The radicles grow out in the direction in which they emerged from the seeds. The seedlings must be kept always moist.

N 2m. Attach a potted plant to a clinostat, and rotate about the stem as a horizontal axis. The direction of growth of the shoot is unaffected by gravity. As a control, leave a similar potted plant lying on its side.

N 2n. Set up some bean seedlings on the face of a wheel rotating rapidly in a horizontal plane (e.g. electrically driven wheel). The radicles grow radially outward—the so-called "centrifugal force" acting in much the same way as gravity.

N 2p. Place 6 beans with short radicles horizontally in saw-dust for 1–2 hours. Mark with indian ink the lower sides of the radicles. Amputate the tips and fix the beans vertically in damp sawdust or on a cork in a jar whose atmosphere is kept damp by wet cotton wool at the bottom. The radicles curve towards the side which was downmost before amputation. Thus the response to the stimulus occurs independently of the tip some time after the stimulus has been received.

N 2q. Demonstrate this delayed response more accurately by stimulating roots by laying them horizontally for one hour, and then placing on the edge of a clinostat with the marked roots lying parallel to the clinostat's horizontal axis.

N 3. Other Tropisms.

N 3a. Rub gently one side of a tendril of white bryony (*Bryonia dioica*) or of a leaf stalk of traveller's joy (*Clematis vitalba*) and record the time which elapses before curvature takes place towards the rubbed side (positive thigmotropism).

N 3b. Hang pieces of cotton of various lengths on a bryony tendril, and observe length necessary to cause curvature. By weighing a length of cotton, the corresponding weight may be estimated. Often as little as 1/100 gm. will suffice.

N 3c. Warm a 3% gelatine solution and place a large drop on a microscope slide. Stir into it some pollen (e.g. of hyacinth, *Scilla nutans*) and also insert the stigma and one or two ovules. Allow to set and keep in darkness in a moist atmosphere for some hours. Upon microscopic examination, the pollen tubes will be seen to have grown in the direction of the stigma and ovules, probably in response to chemicals produced in them (positive chemotropism).

N 3d. Repeat experiment N 3c, but omit stigma and ovules and place a cover glass on the drop. The pollen tubes grow towards the centre of the slide away from the oxygen of the atmosphere (negative chemotropism).

N 3e. Place 10 germinated peas with roots about 1–2″ long, in line abreast in dry sawdust in a flat tray. At one end of the line, keep the sawdust moist by capillary movement of water from a beaker along 4 or 5 cords dipping into it and into the sawdust. Each day uncover the seedlings carefully and observe the direction of the roots. They tend to grow towards the moisture (positive hydrotropism).

N 3f. Fill a shallow tray of perforated metal or metal gauze with damp sawdust. Sow in it 10 soaked peas, and suspend in a warm room. Observe daily. The radicles grow downwards, and then curve up again into the damp sawdust (positive hydrotropism or hygrotropism). Make certain that the spaces in the metal sheet are large enough to allow passage to pea radicles.

See also experiment N 9d.

N 4. Phototaxis.

N 4a. Take a tube containing a sufficient number of *Chlamydomonas* or *Euglena* to colour the water green. Surround it with a paper cylinder with a narrow vertical slit. Stand in a light place for one day and then remove the paper. The organisms will be seen to have gathered towards the light (positive phototaxis). The demonstration may be made particularly striking by using a flat-sided aquarium covered with black paper in which the name of the organism has been cut out in stencil. If the inside of the aquarium is first of all smeared with egg-white, the liquid may be decanted and the green "lettering" may be kept permanently.

N 4b. Place a water-boatman (*Notonecta*), which has been left in the dark for some hours, on a sheet of ground glass in a faintly illuminated dark room, and direct a beam of light across the sheet. The animal crawls towards the light (positive phototaxis). Do not keep out of the water for longer than necessary.

N 4c. Positive phototaxis can be similarly shown with insect

larvae, e.g. caterpillar of yellow tail moth; and in an aquarium with light reaching only one side through a slit, with the water scorpion. Practise is necessary to achieve success.

N 4d. Place some larvae of blowfly (*Calliphora erythrocephala* —"gentles") on a well illuminated flat surface. Shade part of it by a piece of cardboard. As the cardboard is moved, the larvae move to the darkened part (negative phototaxis).

(*N.B.*—As in N 4g.)

N 4e. By using two electric lamps and moving them to varying relative distances and thus altering the relative light-effects on the larvae, the negative phototaxis may be further studied.

(*N.B.*—As in N 4g.)

N 4f. Place blowfly larvae on a moist sheet of ground glass in a very faintly illuminated dark room. Direct two beams of light at right-angles across the glass, so that they cross near the larvae. The larvae move away from the lights, at an angle between the beams depending upon their relative intensities. Thus the negative phototactic response to the two beams gives a "resultant" effect.

N 4g. Place blowfly larvae in a corked test tube, and lay horizontally, at right angles to the window, on a sunny day. Arrange a screen above the tube so that a shadow is thrown on the half nearer the window. Most larvae move away from the window, although this involves going into the more brightly lit half of the tube, i.e. it is definitely light-direction, and not light-intensity, that causes the tactic reaction.

N.B.—Sometimes the reverse may happen (perhaps because the larvae are ready to pupate). Use therefore *young* larvae.

N 4h. Make a cylindrical arena about 5″ high from a long strip of blotting paper. Divide its inner wall into eight equal sectors, and blacken alternate ones. Place some woodlice (*Porcellio scaber*) in the centre. Illuminate from above. The animals move in approximately equal numbers to the four dark areas (it is usually necessary to experiment with

different specimens and arenas of different sizes before success is attained). The reaction is probably negative phototaxis.

See also experiment N 9a.

N 5. Other Taxes.

N 5a. Place a polychaete (e.g. *Arenicola grubei*) in moist sand in a very narrow aquarium (so narrow that the animal can be seen even when burrowing in the sand). It burrows vertically downwards, and when the aquarium is turned onto its side, the animal turns through a right angle, thus once more moving earthwards (positive geotaxis).

N 5b. Place some woodlice in a long wide glass tube, containing a strip of damp paper for a walking-surface, and cork both ends. Slope the tube at about 30° to the horizontal, and observe the accumulation of woodlice at the lower end (positive geotaxis).

N 5c. Place some flatworms (e.g. *Procerodes ulvae*) in a film of water on a sheet of glass. Slope the glass. The flatworms "gallop"—downward (positive geotaxis).

N 5d. Place a millipede (several species may have to be tried before success is attained) on a flat surface near a triangular glass prism. It creeps along against the prism, and when it reaches a corner tends to turn towards the side which was in contact. (thigmotaxis, i.e. movement in a direction determined by that of the line of contact).

N 5e. Dip one end of a strip of wet filter paper into a beaker of water, and bend over edge of the beaker so as to form a steep inclined plane on to the bench. Place on the paper a plasmodium of a slime fungus (myxomycete). It slowly moves up the paper against the flow of water and then down against the current on the paper inside the beaker (rheotaxis). Several days may be required for completion of the experiment.

N 5f. Place some planarians (several stream species may have to be tried before success is attained) in a round glass bowl, and

swirl the water around. Sometimes the animals move against the direction of flow (rheotaxis).

N 5g. Observe fish in a stream. They generally swim against the current, thus remaining roughly in the same place relative to the bank. This "rheotaxis" may be partly governed by an optical fixation of surrounding objects, rather than by a direct reaction to flow.

N 5h. Place a fairly young fern prothallus in water on a slide, and cover with a slip supported on two others. Examine microscopically to make sure that fern sperm are present. Now draw out some heated glass tubing so that it is about 1 mm. in diameter. Seal one end of a section about 1″ long, by holding in the flame for a moment. Put into a small dish of 0·01% malic acid, and place in a vacuum pump chamber. As the air is drawn out of the tube, malic acid enters it. Now insert the open tip of the capillary tube under the cover slip. Microscopic examination shows that sperm move towards the malic acid (chemotaxis or perhaps chemokinesis).

N 5j. Leave a boiled pea to decay in 200 ml. water for 2–3 days. Cover a drop of the liquid on a slide by a cover glass resting on two others. Fill a capillary glass with 2% potassium nitrate solution, and insert its open tip under the cover glass. After about ¼ hour, many bacteria may be observed clustering round the capillary opening, attracted by the nitrate (chemotaxis or perhaps chemokinesis).

See also experiments N 9b, c.

N 6. Orientations.

N 6a. Allow the larva of the small tortoise-shell butterfly (*Vanessa urticae*) to crawl on sooty paper in the dark. It leaves a convoluted path indicating random wandering. Switch on a small source of light to one side of the caterpillar. It now keeps a fairly straight path (photo-orientation).

N 6b. Repeat experiment N 6a with ladybird, millipede, woodlouse, etc.

N 6c. Repeat the second part of experiment N 6a. After a little time, switch off the light and switch on another at the other side of the caterpillar. The caterpillar immediately changes direction, thus maintaining its orientation with respect to light.

N 6d. Repeat the second part of the experiment N 6a. After a period (perhaps a few minutes, perhaps half an hour) the caterpillar changes direction. i.e. the angle of orientation may be altered spontaneously.

N 6e. Lift up a wood ant on a sunny day when on its way back to its "hill" with food, and put it down again a little distance away. It sets off again parallel to the original path, making the same angle with the sun's rays (photo-orientation).

N 6f. Stop the sunlight from reaching the ants returning to the "hill", and throw it on them from the other side by means of a mirror. The ants change direction so as to make the same angle with the reflected rays as they did originally with the direct rays (photo-orientation).

N 6g. Place an aquarium containing *Artemia salina* (brine shrimp) in a dark room and illuminate from above. The animal swims with its ventral surface above. Now move the light below the aquarium. The animal either rolls or somersaults, bringing the ventral surface below (ventral photo-orientation).

N 6h. Repeat experiment N 6g with the carp louse (*Argulus*). (In this case there is a dorsal photo-orientation).

N 6j. Suspend a crayfish in a large aquarium, and observe its swimming movements. Now tilt it sideways. Note that the appendages on the upper side are held stiff, while those on the lower side move in such a way as to re-level the animal (geo-orientation).

See also experiment N 9a.

N 7. Kineses.

N 7a. Divide some woodlice (*Porcellio scaber*) into two equal batches, and place each batch on a fine perforated zinc platform in separate glass vessels. Under one platform have a flat dish of water, and under the other a desiccating agent such as calcium chloride. In the moist chamber most of the animals soon come to rest, while in the dry one they move about actively. This is an example of hygrokinesis i.e. rate of movement is affected by humidity of atmosphere. Remove the animals from the vessels immediately observation has been completed.

N 7b. At one side of a large shallow trough, place some small dishes of distilled water, and at the other of concentrated sulphuric acid. Cover the dishes with a perforated zinc platform, and the trough with a lid or sheet of glass, thus establishing a humidity gradient across the trough. After half an hour pass some woodlice through a small hole in the lid, and re-cover the hole. After about $\frac{1}{4}$–$\frac{1}{2}$ hour, most of the animals will have accumulated on the moister side of the vessel. This is not, as it might appear, a case of hygrotaxis (i.e. directional response to a humidity gradient), but aggregation due to hygrokinesis.

N 7c. Support a large cover slip on two others on a slide, and flood the space underneath with water rich in *Paramecium*. Introduce a drop of "soda water" (i.e. carbonic acid solution) under the cover slip by means of a very fine pipette. Within a minute most of the animals gather around the drop. As the acid diffuses outwards, the circle of animals moves likewise, i.e. they keep within a region of "preferred" or eccritic concentration. This is a chemokinesis, the frequency of turning being altered by the chemical in such a way as to cause aggregation.

N 7d. Repeat experiment N 7c, using first a drop of 0·5% sodium chloride solution and then 0·05% acetic acid solution.

N 7e. Breed a large number of blowflies in a wooden box

having one side made of opaque white blotting paper and the opposite one of black gauze through which the flies may be observed. Place behind the centre of the sheet of blotting paper an electric lamp. The flies collect in an annulus around the lamp (which is invisible to them) in a zone of "preferred" or eccritic temperature. This is a thermokinesis, the activity of the flies being affected by the temperature in such a way as to cause aggregation.

N 7f. Arrange a slide carrying *Paramecium*, with one end surrounded by a water-circulation of about 40° C. and the other of cold water. The animals accumulate at an intermediate position (thermokinesis).

N 7g. Enclose an earwig (*Forficula*) in a petri dish. It presses its body against the wall, attaining maximum contact by becoming curved. This reaction, in which activity is reduced by contact, thus leading to aggregation in crevices etc., is an example of thigmokinesis.

N 7h. Place some sandworms (*Nereis*) in a flat dish moistened with sea water. Add some pieces of glass tubing of about the same diameter on the worms. They "burrow" into the tubes (thigmokinesis).

See also experiments N 5h, j; N 9b, c.

N. 8. Nasties.

N 8a. Cover a pot of daisies with an opaque box and leave for some time. The flowers "close", and re-open when the box is removed (photonasty).

N 8b. Repeat experiment N 8a with wild or garden *Oxalis*. The leaves fold in the dark and open in the light (photonasty).

N 8c. Alter the temperature surrounding a pot of tulips. With rising temperature the flowers open, with falling temperature they close (thermonasty).

N 8d. Hold a lighted match beneath a terminal pair of leaflets of the sensitive plant (*Mimosa pudica*). Observe the order in

which the leaflets close (thermonasty)—from the tip of that leaf, and then towards the tip of the other leaves, as if the movement were due to the passage of a chemical in the sap from the irritated leaflets.

N 8e. Very carefully touch with a fine point, the inner face of the filament of a stamen of the common barberry (*Berberis*). The stamens at once move inwards (thigmonasty or perhaps seismonasty).

N 8f. Touch the stigma of a monkey flower (*Mimulus*). The two flat lobes, normally spread apart, close together (thigmonasty or perhaps seismonasty).

N 8g. Touch a leaflet of the "sensitive plant", *Mimosa pudica*. The neighbouring leaflets fold up, and the effect of the stimulus gradually spreads through the plant (thigmonasty or perhaps seismonasty).

N 8h. Tie a thread to a branch of *Mimosa*, and attach the other end to the pendulum of a metronome, making about 10 beats per minute. The leaflets fall, but eventually rise again, and are then insensitive to disturbance for some time, owing to excessive stimulation.

N 8j. Hold a bottle of concentrated ammonia solution near a plant of *Mimosa*. The leaflets gradually close (chemonasty).

See also experiment N 9d.

N 9. Complex Reactions.

N 9a. Place some *Daphnia* in an aquarium in a dark room, and illuminate from above. The animals swim up towards the light, with the back obliquely upwards. Now illuminate from below. They swim downwards with the dorsal surface facing downwards. This is a combination of positive phototaxis with a dorsal photo-orientation.

N 9b. Place a small freshly cut piece of ox liver in the centre of a circular glass dish 10 cm. in diameter, containing *Planaria lugubris* (a pond form). After a little while, the head is raised

and waves slowly, and then crawling begins along a very convoluted path. This movement is probably a chemokinesis, i.e. a reaction to intensity of chemical stimulation. When the planarian gets nearer the bait, it moves more directly towards it. This is probably a chemotaxis, i.e. a reaction to the direction of the chemical gradient.

N 9c. Expose a piece of meat in summer on a large table in the open. Probably blowflies (*Calliphora erythrocephala*) will soon appear in the neighbourhood. At first their movements form a convoluted track (probably a chemokinesis) and as they approach the meat a fairly direct track (probably a chemotaxis).

N 9d. Carry out experiments on the reaction of the sundew (*Drosera rotundifolia*) tentacles to pressure, water, insects, hard-boiled egg white, etc. It is difficult to decide whether the movement is a tropism or a nasty and it appears to have both thigmo- and chemo- components.

See also experiment N 5g.

TOPIC P.

NERVOUS SYSTEM

In animals, sensitivity is dependent mainly on the nervous system. In simple forms, a nerve-net without any controlling centre is diffused through the organism. Thus parts of the animal can function even when separated from it. In higher forms, concentration into definite centres occurs. This allows greater co-ordination and complexity of action, but reduces the independence of the separate parts of the nervous system.

A nerve appears as a whitish glistening thread, and is made of many fibres, each of which consists of a central protoplasmic core arising from the main body of a nerve cell (neurone), a myelin sheath of fatty material, and a membranous neurilemna sheath. The nerve fibres transmit electrical impulses which in some way signalise sensations.

In a reflex action, a receptor cell receives a stimulus, a nervous impulse passes along a fibre, across synapse(s) to one or more other fibres, and finally the impulse reaches an effector cell. In worms and arthropods the neurones are concentrated in the "nerve cord", in vertebrates in the spinal cord and brain (the central nervous system).

The simplest reflex actions in vertebrates involve merely the path:—receptor → afferent fibre → spinal cord → efferent fibre → effector. In other cases, the reflex action is modified by the action of various parts of the brain. Those reflexes involving only spinal cord and hind- and mid-brain are innate or "unconditioned" (e.g. swallowing, knee-jerk, blinking). It is however, possible for many animals to develop other more complex reflexes, involving the higher centres of the brain. These are the so-called acquired or "conditioned" reflexes. These conditioned reflexes are the basis of learning.

One of the results of the continual receipt of messages by the brain is that some areas of it become inhibited. Eventually this inhibition spreads over larger and larger regions of the brain, until an important part of it is out of action. This

widespread inhibition manifests itself as sleep, not only in mammals and birds, but also to a certain extent in some other animal groups such as fish. Hypnosis is a similar phenomenon.

Commonly people speak of "the five senses"—vision, hearing, touch, taste and smell. Actually the last two are closely related, while there are other skin senses such as heat, cold, and pain in addition to touch. Then there are also the senses of hunger and thirst, the sense of balance, and those muscular senses which allow us to gauge the position of our limbs and to co-ordinate our movements. Not all these senses are present in all animals, and their relative importance varies in different groups.

The sense of sight may range from a vague generalised awareness of light (as in the earthworm), to the perception of brightness, direction, movement, form and colour (as in humans). The receptor organ may be a simple "eye-spot" (as in many protozoa), a series of isolated photosensitive cells (as in the earthworm), an optic cup with retina (as in some worms, molluscs and arthropods), a closed optical vesicle with retina, vitreous humour, lens, etc. (as in some gastropods), or an elaborate eye with retina, vitreous humour, lens, cornea, eyelids, accommodating muscles, variable iris, etc. (as in the higher vertebrates and cephalopods). Another line of development is that of the faceted eye (as in insects and crustaceans).

The simplest organisms can perceive only intensity or direction of light; many insects, molluscs, amphibia, etc. perceive the movement of objects but cannot distinguish their form when stationary; bees, mammals, birds and perhaps some reptiles and cephalopods can perceive form also; while colour is distinguished over various ranges and with varying degrees of sensitivity by these latter groups and perhaps some others.

The sense of hearing (i.e. the perception of pressure waves in the atmosphere) occurs in quadrupeds, birds and some insects. Usually in the former the sense organs are in the head, and in the latter on the body or legs.

In all cases a tympanum receives the vibrations, and in most insects the sensory cells are directly attached to it. In the

green grasshopper however, the vibrations are transmitted to the sensory cells *via* an intermediate mechanism, as in quadrupeds.

It is difficult to draw an absolute distinction between hearing and the perception of pressure changes in other media such as water—a sense possessed by fish and many other water animals.

The so-called "sense of balance" is in fact not a single sense. Either by simple statocysts (as in crustaceans, etc.) or by liquid in the semi-circular canals of the ears (as in vertebrates), equilibration is normally achieved. It is however, clear that many animals also utilise vision as an aid to balance, while sometimes the suspension of the viscera provides information about balancing.

The skin senses are those of touch and pressure, heat and cold, and pain. The sensation of pain is aroused when any of the normal stimulants passes a certain threshold intensity. Pain is sensed by end-organs consisting of terminal arborisations of nerve fibres, while the other senses have as their receptors nerve fibre end-organs which are encapsuled. In general, the various types of end-organs are correlated in their distribution on the skin, being all frequent in some parts and all rare in others, but there are exceptions to this rule.

Sensitivity to chemicals has three aspects—the irritant, the olfactory and the gustatory. In higher animals such as humans, these aspects are fairly distinct and have their own distinct nervous mechanisms. Thus the trigeminal nerve of the nasal chamber detects irritants, the olfactory nerve of the uppermost part of the nasal chamber detects more delicate perfumes, while the taste buds of the mouth detect tastes.

This triple distinction is not complete even in humans, and in many of the lower organisms only a generalised "chemical sense" can be recognised. In worms, crustaceans, gastropods, etc. the olfactory and gustatory components cannot easily be distinguished. The cells sensitive to chemical stimuli occur often over large areas of the skin (e.g. the starfish's thousands of "feet", the "suckers" of cephalopods). Insects possess

definite organs of smell and taste. The former are on the antennae, and are often of remarkable sensitivity. The latter are usually in the mouth and on the palps, but sometimes on the feet. It has been shown that bees have a sense of smell very similar to that of humans.

In humans, separate taste buds are specially sensitive to sour, salt, bitter and sweet tastes and are concentrated at the sides, sides and tip, base, and tip of the tongue respectively. Some substances can stimulate two types of bud, and thus taste both sweet and bitter. While many substances have both taste and smell, others affect only one component of the chemical sense. In normal appreciation of food, "taste" and "smell" are surprisingly intermingled.

Experiments are classified as follows:—

P1. Primary Nerve-net.
P2. Unconditioned Reflex.
P3. Conditioned Reflex.
P4. Learning.
P5. Inhibition.
P6. Vision
P7. Hearing.
P8. Chemical Senses
P9. Skin Senses.

P 1. Primary Nerve-net.

P 1a. Decapitate a flatworm (*Turbellaria*) and allow it to rest for a little while. Eventually, on gentle stimulation, normal locomotion occurs, being governed by a primary nerve-net.

N.B.—The experiment does not *prove* that a primary nerve-net is involved.

P 1b. With clean scissors cut off a tentacle of an anemone (*Anemonia*) fairly near the base, place it in a dish of clean salt water and allow to rest for 10 minutes. Now touch the tentacle —it responds as when it was attached to the animal.

P 1c. Flood the tentacle with a suspension of crushed anima

matter in salt water. Movements take place, of such a nature as to drive the food towards the side on which the food entrance originally was.

P 2. Unconditioned Reflex.

P 2a. Sit on a stool with one leg crossed over the knee of the other. Strike the crossed leg smartly with the edge of the hand, just below the knee-cap. Repeat until a spot is found, the hitting of which causes your foot to jerk forward—a reflex action.

P 2b. Pass a pencil lightly over the sole of the foot. There is a reflex movement of the toes.

P 2c. Ask your partner to close the eyes for a couple of minutes to allow them to become accustomed to the dark, and then to open them. Now shine a torch into the eyes. The pupils contract in size—a reflex action.

P 2d. Repeat experiment P 2c, shielding one eye by means of a piece of cardboard, and shining the light into the other. Remove the cardboard and observe the co-ordinated action of the two pupils.

P 2e. Lightly anaesthetise a frog by a mild dose of chloroform, and then decapitate with a pair of large scissors. (Alternatively the brain may be destroyed by "pithing", but this is a much more difficult operation.) Allow the frog to recover for an hour, and then squeeze the toes. The legs withdraw—a spinal reflex.

P 2f. On the flank of the same frog, place a little dilute acid. The frog moves its leg in such a way as to rub off the acid—another spinal reflex.

P 2g. Pass a needle down the spinal column to destroy the spinal cord, and repeat experiments P 2e, f. There is now no reflex movement.

P 2h. Touch an earthworm on the head. There is a violent

contraction of its longitudinal muscles—a "protective reaction."

P 2j. Set up a colony of the barnacle (*Balanus*) usually found attached to fresh mussels. Place in sea water in a glass aquarium in fairly bright light. Allow it to rest for some time until it has settled down in its new environment. Then allow a shadow to fall across it. The animal retracts its limbs and closes up (the "shadow reflex").

P 3. Conditioned Reflex.

P 3a. Shortly before feeding goldfish, tap the aquarium on each occasion always at one particular corner. Eventually a conditioned reflex is established and the fish gather in the corner in response to tapping alone, without any food being presented.

P 4. Learning.

P 4a. Place a sand-hopper (*Gammarus*) on the palm of your hand. It feigns death. Time with a stop-watch the number of seconds before it leaps away. Repeat ten to twenty times. The period of feigning gradually diminishes with experience.

P 4b. Similarly investigate several times in succession how close a finger may be brought to a snail's eyes before it draws them in.

P 4c. All members of the class hold hands in a circle, leaving a single gap. Stand in the gap, and squeeze with your left hand the right hand of the person on your left. In the right hand hold a stop-watch and start it at the same moment. Each member of the ring squeezes with his left hand as soon as he feels his right hand being squeezed. Transfer the watch to the left hand. Stop the watch the moment your right hand is squeezed by the person on your right. Divide the recorded interval by the number of members in the ring, thus calculating the mean reaction time. Repeat several times and notice how reaction time decreases with practise.

P 5. Inhibition.

P 5a. Observe the muscular waves over the foot of a slug or snail, controlled by a nerve-net. Touch the head gently, or tap the containing vessel. Movement ceases for a while, owing to inhibition of the waves by the cerebral ganglion.

P 5b. Suspend a starfish (*Asterias*) in a cotton sling. The tube feet are violently active. Apply glass slips—activity ceases when contact is established. In this case, activity controlled by a primary nerve-net is inhibited by the normal contacts of the tube feet.

P 5c. Repeat experiment P 5b with the legs of an inverted cockroach and a suitable surface, such as a microscope slide.

P 5d. Sit down in a comfortable chair with the left arm extended, wrist uppermost. Let your partner feel with finger tips the pulse of the exposed wrist, and count the number of beats per minute. Now at a sign from your partner, drink slowly but fairly continuously a large glass of water. After drinking has continued for about ½ minute, your partner counts the number of pulse beats per minute while drinking still continues. There is a decrease of pulse rate, since the act of drinking causes inhibition of the muscular contractions of the heart.

P 6. Vision.

P 6a. Dissect out a fresh bullock's eye. Cut a small window in the back of the eye and cover with translucent paper. Darken the room, and hold a light before the eye. An inverted image appears on the paper. If the eye of an albino animal is used, there is no need to cut out the window.

P 6b. Dissect further, separating carefully the lens. Place it on a piece of newspaper. The type is magnified.

P 6c. Press with the fingers against the balls of the closed

eyes. Coloured patterns appear. Since the normal function of the eyes is vision, the brain becomes conscious of "light sensations" although the exciting agent is pressure.

P 6d. Close the left eye, and hold about 18″ away a white card bearing a black cross and black spot about ¼″ each in size, separated by about 2½″. Keep your gaze fixed on the spot (at left side of card) and bring the card gradually towards the eye. Out of the corner of the eye observe the cross. At a certain point the cross becomes invisible, since its image is being cast upon a "blind spot" of the retina.

P 6e. Place on the bench a sheet of white paper, about 6″–8″ square. At the left side place a block about 6″–8″ high, holding the paper in place. Rest your forehead on the block, and close the left eye, looking fixedly with the right eye at a cross marked on the paper just to the right of the block. Move a long pencil with a fine point from right to left towards the cross. Make dots where the point disappears from vision, and again where it reappears. Repeat, moving the pencil at right angles (i.e. from top of paper to bottom) and in other directions. Thus map out the "blind spot" in the field of vision. If this area is shaded black, it will be obvious that it should vanish from view when the head is placed in the same position, and the right eye focussed on the cross. This experiment requires some degree of concentration and patience, for there is always a tendency to *look* at the point of the pencil instead of at the cross.

P 6f. Make a pinhole in a piece of card and hold it about 1″ in front of the right eye, keeping the left eye closed. A blurred circle of light is seen, surrounded by darkness—the shadow of the iris. Now open the left eye. The circle of light contracts, owing to the extra illumination on the left eye causing a co-ordinated contraction of both irides.

P 6g. Make a pinhole in a piece of card and hold it about 1″ in front of the eye. Now move the head of a pin in front of the eye, just touching the eye lashes. It is so close to the eye

that its lens forms an erect shadow of the pin head on the retina, and so the pin head is seen inverted.

P 6h. Attach short strings to holes made at opposite edges of a piece of white card about 2″ square. In the centre of one side of the card draw a bird cage, and in the centre of the other side a bird. Twirl the card rapidly on its strings, when the bird is "seen" in the cage. This demonstrates persistence of retinal impression.

P 6j. Look at a candle flame in the dark, and then blow it out. The impression is received that the candle can still be seen for a fraction of a second. This is the positive after-image. It is followed by a very black patch—the negative after-image.

P 6k. Look at a red square fixedly for some time, and then at a white surface. A greenish square is seen. This demonstrates successive colour contrast.

P 6l. Draw a 1″ square on a white card and divide into a hundred squares of size 1/10″. Now with one eye only open, make 100 quick stabs with a pencil at preselected squares. Record the percentage of successes. Repeat using both eyes.

P 6m. Investigate the response of an earthworm to light focussed by means of a hand lens on various parts of its body. Especially near the head it is sensitive to light, but there is no evidence of appreciation of form, etc.

See also experiments P 2c, d.

P 7. Hearing.

P 7a. Hold a watch near your partner's ear. Move away slowly, and record the maximum distance from which the watch can still be heard. Now change places with your partner. In this way the relative sensitivities of hearing of all members of a class may be determined.

P 7b. Test with a Galton whistle the highest note which can be heard by various members of the class.

P 7c. Plug your ears with cotton wool or rubber ear plugs, until the tick of a watch can no longer be heard. Now place the watch between the teeth, against the forehead, etc. The tick can now be heard, owing to the conduction of vibrations through the bones of the head.

P 7d. Place some earthworms on the surface of soil in a plant pot. Play a piano or gramophone loudly in the room. The worms seem unaffected by the sound.

P 7e. Repeat experiment P 7d, but with the pot actually on the musical instrument. The worms bury themselves in the soil, indicating sensitivity to the vibration.

P 8. Chemical Senses.

P 8a. Blindfold your partner, and experiment with grains of sugar, grains of salt, quinine solution, and vinegar, thus finding which regions of the tongue and the back of the mouth are sensitive to sweet, salt, bitter, and sour tastes respectively. The grains can be placed on the tongue by forceps, and the solutions by a glass rod.

P 8b. Make an infusion of cloves by boiling one or two for a few minutes in a pint of water, and allowing to cool. Blindfold your partner, who tries to distinguish between drops of cloves and of distilled water, placed on the tongue in haphazard succession by means of a glass rod. Keep a record of the percentage of correct interpretations. Repeat with the partner's nose closed by a clip. There is a much lower percentage of correctness, since much of what appears to be taste is really smell.

P 8c. Dissolve 1 gm. sucrose in 100 ml. distilled water, and make up by dilution solutions ranging in concentration from 1% downwards. See how dilute a solution your partner can detect, applying the same safeguards (blindfolding, distilled water, haphazard succession) as in experiment P 8b.

P 8d. Chop up an onion and an apple. Test as in P 8b, the

ability to discriminate between them by taste-plus-smell (nose open) and by taste only (nose clipped).

P 8e. Apply to the tongue the terminals of a 4-volt electric cell. The electric current stimulates the taste nerves and thus a sensation of "taste" is experienced.

P 8f. Roll three pieces of cotton wool into compact balls, and to each add a length of thread. Soak one in sugar solution, and one in a mixture of saliva and salt water. "Feed" each of the 3 pieces in turn to an opelet anemone (*Anemonia sulcata*) in an aquarium. The saliva-soaked ball is accepted, but the other two rejected.

P 8g. Place soil in several plant pots. About ¼" below the surface, bury small pieces of onion, cabbage leaf, etc. On each pot place one or two earthworms. They tend to burrow fairly quickly and to devour the food. The results seem to indicate some sense of smell. As a control, set up pots without hidden food.

P 8h. Scatter about on the surface of soil in a box, small pieces of meat, bread, and cabbage, onion, celery, parsnip, beetroot and carrot leaves (ten of each). Place earthworms on the surface, and leave for some weeks. Now observe how many pieces of each kind have been partly eaten. The results seem to indicate some taste preference on the part of the worms.

P 8j. Bring a glass rod dipped in alcohol or xylol near the head of a snail. The shorter "feelers" withdraw, indicating a sense of smell. Carry out a control experiment with a plain glass rod.

See also experiments G 1d; P 1c; P 2f.

P 9. Skin Senses.

P 9a. Blindfold a friend and find the smallest piece of paper which can be felt when dropped from a height of 6" on to the palm of the hand. Repeat on to the forehead. The skin of the forehead is found to be the more sensitive. Test ten times with each piece of paper, and take the result for which the correct answer is given eight times.

P 9b. With variable dividers, test on a blindfolded friend the smallest distance between the points necessary for them to be separately distinguished as two points, on various parts of the skin. Test ten times for each distance, and take the result for which the correct answer is given eight times. Suitable places to test are:—tip of tongue, tip of forefinger, tip of nose, palm of hand, back of hand, back of neck.

P 9c. Blindfold a friend, and with a hot knitting needle test the reaction at various points on the skin of the forearm. Mark with ink the points where heat as distinct from mere pressure is reported. In this way, map the "heat spots" of the skin. The needle is conveniently heated in a beaker of hot water.

P 9d. Similarly with a really cold knitting needle test and map the "cold spots", marking in a different colour. The needle is conveniently cooled in a beaker containing a freezing mixture.

P 9e. Similarly with a fine stiff bristle, test and map the "touch spots".

P 9f. Similarly with a fine sterilised needle, test and map the "pain spots".

P 9g. Rub a piece of menthol on the skin. This chemical stimulates the "cold spots", thus giving the sensation of coldness.

See also experiments P 2b, h.

TOPIC Q.

REPRODUCTION, VARIATION AND HEREDITY

It is difficult to draw a really sharp division between regeneration and asexual reproduction. Many of the lower animals and most plants have the capacity of replacing lost organs, and this capacity grades into the type of asexual (vegetative) reproduction exemplified by budding and the growth of cuttings. Other types of asexual reproduction however, (e.g. reproduction by fungal spores) are very far removed from simple regeneration.

The essence of sexual reproduction is the growth of a new individual as a result of the fusion of two cells. These cells may be almost or quite identical (e.g. some *Chlamydomonas spp.*), but typically are ova (larger, more stationary, female) and spermatozoa (smaller, more motile, male). The male and female cells may be from the same (self-fertilising) or different (cross-fertilising) individuals. When cross-fertilisation occurs, the individuals may be unisexual (producing either ova or sperm) or hermaphrodite (producing both ova and sperm). In many cases it is known that sperm are attracted to ova by chemicals (e.g. malic acid in ferns).

In the study of pollination, the school garden should be utilised to the utmost extent in large scale experiments. Care should be taken to distinguish sharply between pollination and fertilisation, and the rôle of insects in pollination should be studied.

Heritable variation within a species may be of several different types. There is for example, variation in a character inherited in the cytoplasm of the egg cell (e.g. green and yellow races of *Primula sinensis*, depending on type of chloroplast); variation due to a single nuclear gene (e.g. normal and "fern-leaved" *P. sinensis*); variation due to several genes (e.g. stem-colour in *P. sinensis*, height in humans); variation due to rearrangement of genes (e.g. many characters in *Drosophila*);

and variation due to the presence of extra sets of chromosomes (e.g. diploid and tetraploid tomatoes).

A great deal of variation however, is not hereditary, but is due to environmental factors. This is the case with many of the "modifications" upon which Darwin (falsely) based his ideas of natural selection. Within a pure line of beans, only "environmental" variation is effective, but in an ordinary garden population the variation will be partly due to environment, partly due to heredity. Metrical characters particularly lend themselves to statistical consideration. The "normal curve" should be derived from such measurements, being presented in block form as well as in a continuous curve.

Simple experiments in heredity may be carried out on garden plants and on pets, although it is often convenient to use quick-breeding animals that may be kept in the laboratory. While the ideas of dominance, recessiveness, genes, etc. must be introduced, care should be taken from the first not to give an over-facile and too mechanical interpretation of the terms.

Experiments are classified as follows:—

Q1. Regeneration and Asexual Reproduction.
Q2. Sexual Reproduction.
Q3. Variation.
Q4. Heredity.

Q 1. Regeneration and Asexual Reproduction.

Q 1a. Cut off the tentacles from a living *Hydra* and observe the gradual regeneration of new ones.

Q 1b. Record by description, drawings and photographs, the gradual healing of a wound of a tree caused by the lopping of a branch.

Q 1c. Cut some planarians into pieces of various sizes, making transverse, oblique and longitudinal cuts in different specimens. Leave under favourable conditions with a good food supply, and observe the gradual regeneration of complete bodies.

Q 1d. Cut a dandelion root into several pieces, and plant in moist soil in the spring. After some weeks, each piece develops into a new plant.

Q 1e. Similarly grow cuttings of willow, geranium, etc.

Q 1f. Cultivate *Mucor* spores from mouldy bread as in experiments Q 2e, f.

Q 1g. Carefully remove the complete cap from a mature mushroom, and place gill downwards on a sheet of white paper. Remove carefully after a few hours, when the spores will be seen on the paper in dark radiating "spokes". Such a pattern may be made permanent by using waxed paper, and warming slightly at the end of the experiment to melt the wax, and then allowing it to cool and set again.

See also experiment M 5c.

Q 2. Sexual Reproduction.

Q 2a. Examine "hard roe" (ovaries) and "soft roe" (testes) of herring.

Q 2b. Kill an earthworm by placing in alcohol, and dissect out the seminal vesicles. Place the contents in 0·75% salt solution, and examine microscopically the motile sperms. (Do not confuse with parasites). Alternatively obtain sperm from the seminal vesicle of a male frog in the spring.

Q 2c. Mount in water a mature fern prothallus. Place on a slide the part containing male reproductive organs. The sperm may be observed swimming about.

Q 2d. Collect some mature bladder wrack (*Fucus vesiculosus*) plants and allow them to dry out slightly. Some will exude an orange slime containing antheridia and others a green slime containing oogonia. Mount each in drops of sea water and examine microscopically, when ova and sperm may be seen. If the two are mixed, fertilisation can sometimes be observed.

Q 2e. Make up some test tubes with 2, 4, 6, 8, 10% sugar solution respectively. Place a drop of each on separate micro-

scope slides and to each add a few grains of pollen. Place the slides in moist atmosphere under a bell-jar, and examine after a few hours. Probably at least one of the slides will show the growth of pollen tubes.

Q 2f. Repeat experiment Q 2e, placing pollen and sugar solution on cover slips, which are then inverted over rings of plasticine on slides, thus providing their own "miniature bell jars". This is the so-called "hanging drop" method.

Q 2g. Excise the stamens from 50 unripe flower buds of bluebell, buttercup, snapdragon, or other suitable plant, and enclose the buds in muslin bags. Repeat with 50 other flower buds, but to them transfer pollen from other flowers of the same species. Observe from the numbers of seeds set in the two groups that pollination is necessary to seed formation.

N.B.—The experiment is so devised that the wounding effect of excision is counterbalanced in the two groups.

Q 2h. Enclose 100 unripe flower buds in muslin bags, thus excluding insects, etc. Observe the percentage of cases in which seed are set. Typical figures are:—foxglove, broom, 0%; dwarf bean, pea, 100%; hollyhock, marsh marigold, 50%.

Q 2j. The class splits into groups or individuals, each observing for about an hour, one plant or clump of one species, in a botanic garden or natural habitat. By combining the class results an estimate can be made of the frequency with which different species of plants are visited by bees, wasps, flies, beetles, butterflies and moths.

See also experiment M 3g.

Q 3. Variation.

Q 3a. Measure to the nearest mm. the lengths of not less than 100 broad beans. Place in separate piles all those of lengths (a) mm., (a+1) mm., (a+2) mm., etc. Place the beans of each pile in a wide test tube, so that the heights of beans in tubes will roughly represent numbers of beans with particular lengths. Stand the tubes in a row in order, when a "normal curve" of variation of bean length will be visually apparent.

Q 3b. Count the number of beans in each class in experiment Q 3a and plot length of bean against number of beans having that length. A "normal curve" is obtained. Similarly make a block graph, in which instead of each class being represented by a point, it is represented by a rectangle whose height is proportional to the number of beans in the class.

Q 3c. Similarly measure and record graphically the diameters of snail shells, breadth of crab carapaces, lengths of runner bean fruits, etc.

Q 3d. Similarly record graphically the heights of a human population. Typical figure for British men are:—

Height in inches	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76
No. with that height	1	1	5	9	20	46	78	115	143	155	143	124	75	46	24	9	3	2	1

Q 3e. Similarly count and record graphically the numbers of ray florets in daisy heads, marigold heads, etc.; numbers of "stalks" in umbels of parsley, etc.; numbers of stigma rays in heads of poppy, etc.

Q 4. Heredity.

Q 4a. Cross pure-bred peas having yellow cotyledons with pure-bred peas having green cotyledons. All offspring have yellow cotyledons—a "dominant" character. Only a small section of the seed coat must be removed to observe cotyledon colour, otherwise the seed may not germinate. It is useful to use types such as "British Queen" which has yellow cotyledons visible through a white seed coat, and "Eclipse" with green cotyledons similarly visible, thus rendering unnecessary any removal of the seed coat.

Q 4b. Sow seeds obtained in experiment Q 4a, and record the cotyledon colour of the seeds of the resulting plants. The proportion of yellow: green is approximately 3 : 1 if several hundred seeds are examined.

Q 4c. Other dominant-recessive pairs in peas which may be used for similar experiments are: purple flowers—white flowers, glaucous pods—non-glaucous pods, normal leaves with tendrils—leaves without tendrils.

Q 4d. In tomatoes, the redness of "Fireball" is dominant to the yellow colour of the fruits of "Golden Queen". Note however, that in this as in many other apparently simple cases of dominance, more than one gene pair is involved.

Q 4e. Pure-bred albino Angora rabbits may be crossed with pure-bred Belgian Hares. Normal hair is found to be dominant to angora hair, and normal colour dominant to albino.

Q 4f. In *Gammarus chevreuxi* (brackish water shrimp), simple experiments may be carried out on the inheritance of eye colour. Normal eye has black facets on a white ground. Three recessive genes produce red facets, no facets, and no white ground respectively. Dominance may be readily demonstrated. The sexes may be distinguished at maturity by the easily visible ovary of the female. New broods are produced every 12 or so days, and may be examined by pipetting them on to a slide and placing under a low power objective, preferably using reflected light.

Q 4g. In *Drosophila melanogaster* (vinegar fly) normal wing colour is dominant to "dusky" wing, and red eye to light eye. A new generation is produced approximately each fortnight, and may be examined with a low power microscope.

Q 4h. "Incomplete dominance" can be shown by crossing red snapdragons with white, the resulting offspring being of an intermediate shade. In the second generation a whole series of shades from red to white is obtained.

Q 4j. Chromosomes may be demonstrated quite simply by Belling's "squash" method. Tease out the tissue (e.g. anther, insect testis) in aceto-carmine on a slide with iron needles, remove the larger debris, and apply a cover slip. Heat gently over a small flame for a few seconds and observe microscopically.

Q 4k. Place 50 black marbles (representing gametes carrying a gene *A*) and 50 white ones (representing gametes carrying an allelomorph *a*) in each of two boxes. Label one box "ova" and the other "spermatozoa", and shake to mix thoroughly. Now prepare a score sheet with three columns headed *AA*, *Aa* and *aa* respectively. Pick one marble at random out of each box, and enter a mark under the relevant column, according as the two marbles picked are both black (*AA*) one black and one white (*Aa*) or both white (*aa*). Repeat a large number (at least 100) times, returning the marbles to their respective boxes and shaking well after recording each result, so that the conditions of selection remain unaltered. Add up the score, when a ratio of approximately 1 : 2 : 1 is obtained for the three columns. This represents the effect of random mating in a population heterozygous for a gene pair. If *A* is dominant to *a*, the ratio of the phenotypes *A* and *a* becomes 3 (i.e. *AA*+*Aa*) : 1 (i.e. *aa*).

Q 4l. Repeat experiment Q 4k, but have in each of one pair of boxes, 50 black marbles (*A*) and 50 white (*a*); and in each of two other boxes, 50 red (*B*) and 50 blue (*b*); representing two gene pairs (*A*, *a* and *B*, *b*). Pick one marble from each box on each occasion. The score-sheet will need 9 columns (*AABB*, *AABb*, *AAbb*, *AaBB*, *AaBb*, *Aabb*, *aaBB*, *aaBb*, *aabb*), and the scores will be approximately in the ratio 1 : 2 : 1 : 2 : 4 : 2 : 1 : 2 : 1. This represents the effect of random mating in a population heterozygous for two gene pairs. If *A* and *B* are dominant to *a* and *b* respectively, the phenotypes *AB*, *Ab*, *aB*, *ab* are in the ratio 9 (i.e. *AABB*+*AABb*+*AaBB*+*AaBb*) : 3 (i.e. *AAbb*+*Aabb*) : 3 (i.e. *aaBB*+*aaBb*) : 1 (i.e. *aabb*).

TOPIC R.

TECHNIQUE

The only way really to improve technique is constantly to practise it. It is however, possible to give a few hints to guide the practice into profitable channels.

Hints are classified as follows:—

R1. Laboratory Recipes.
R2. Collection and Culture Methods.
R3. Preservation of Plant Specimens.
R4. Aquarium Management.
R5. Use of Microscope.
R6. Plant Sectioning.
R7. Illustrative Models.

R 1. Laboratory Recipes.

R 1a. *Aceto-carmine.* Mix 45 ml. glacial acetic acid and 55 ml. distilled water. Heat to boiling point and add 0·5 gm. carmine. Shake and filter when cool.

R 1b. *Artificial Sea-water.*

450 gm. sodium chloride
35 gm. magnesium sulphate
50 gm. magnesium chloride
20 gm. potassium sulphate

Dissolve in 20 litres distilled water.

R 1c. *Benedict's Qualitative Solution.*

A. 173 gm. sodium citrate
90 gm. anhydrous sodium carbonate

Dissolve in 850 ml. distilled water and filter.

B. 17·3 gm. copper sulphate crystals

Dissolve in 150 ml. distilled water.

Add solution B to A slowly, with constant stirring. The solution does not deteriorate on standing.

R 1d. *Buffer Solutions.*

Solutions of known acidity or alkalinity are often useful for standardisation (e.g. with colorimetric pH indicators). The following standards are simple ones:—

pH 2 —	10 ml.	N. hydrochloric acid		
pH 3 —	10 ml.	33% acetic acid		
pH 4 —	27 ml.	,,	+ 5 gm.	sodium acetate
pH 5 —	5 ml.	,,	+10 gm.	,,
pH 6 —	100 ml.	$\frac{M}{5}$ potassium dihydrogen phosphate	+11.4 ml.	$\frac{N}{5}$ sodium hydroxide
pH 7 —	100 ml.	,,	+59.4 ml.	,,
pH 8 —	100 ml.	$\frac{M}{5}$ boric acid	+ 8 ml.	,,
pH 9 —	100 ml.	,,	+42.6 ml.	,,
pH 10—	100 ml.	,,	+88 ml.	,,

In each case make up to 1 litre with distilled water.

R 1e. *Culture Medium (Liquid, for yeasts—modified Pasteur)*

1.0 gm. potassium dihydrogen phosphate
0.1 gm. calcium monohydrogen phosphate
0.1 gm. magnesium sulphate
5.0 gm. ammonium tartrate
75 gm. glucose

Dissolve in 1 litre distilled water.

R 1f. *Culture Medium (liquid, for algae—Benecke)*

0.5 gm. calcium nitrate
0.1 gm. magnesium sulphate
0.2 gm. potassium dihydrogen phosphate

Dissolve in 1 litre distilled water, and add a trace of ferric chloride.

R 1g. *Culture Medium (liquid, for bacteria—Bouillon)*

Mince 500 gm. lean meat, and mix thoroughly with 1 litre water. Keep in a cool place for 12 hours, stirring occasionally.

Filter the mixture through fine muslin and make up to 1 litre with water. Add 10 gm. peptone and 5 gm. sodium chloride. Autoclave for ¼ hour or heat in a steam oven for ½ hour, to sterilise. Decant the liquid, and neutralise with sodium hydroxide to pH 7.4.

R 1h. *Culture Solutions (for plant growth).*

Complete—	0·70	gm.	KNO_3
	0·25	gm.	$CaSO_4 2H_2O$
	0·25	gm.	$CaHPO_4\ 2H_2O$
	0·25	gm.	$MgSO_4 7H_2O$
	0·08	gm.	NaCl
	0·005	gm.	$FeCl_3 6H_2O$

Dissolve in 1 litre distilled water.

Add 1 ml. of 0.06% boric acid solution and 1 ml. of 0.04% manganese chloride solution.

Lacking	potassium -	replace	KNO_3	by 0.59 gm. $NaNO_3$
,,	calcium -	,,	$CaSO_4 2H_2O$	by 0·20 gm. K_2SO_4
			$CaHPO_4\ 2H_2O$	by 0·71 gm. $Na_2HPO_4 12H_2O$
,,	nitrogen -	,,	KNO_3	by 0·52 gm. KCl
,,	phosphorus-	,,	$CaHPO_4\ 2H_2O$	by 0·16 gm. $Ca(NO_3)_2$
,,	sulphur -	,,	$CaSO_4 2H_2O$	by 0·16 gm. $CaCl_2$
			$MgSO_4 7H_2O$	by 0·21 gm. $MgCl_2$
,,	magnesium-	,,	$MgSO_4 7H_2O$	by 0·17 gm. K_2SO_4
,,	iron -	- omit	$FeCl_3 6H_2O$	

The salts used should be of very high purity, to ensure that no other salts are present unbeknown.

R 1j. *Fehling's Solution.*

A. 35 gm. copper sulphate

Dissolve in 1 litre distilled water.

B. 173 gm. sodium potassium tartrate ("Rochelle salt")
120 gm. sodium hydroxide
Dissolve in 1 litre distilled water.

Mix equal volumes of solutions A and B shortly before use, since the mixture deteriorates on standing.

R 1k. *Guaiacum Tincture.*

Scrape a lump of guaiacum resin and dissolve the inner part in 95% alcohol (making a 1.5% solution) by heating in a flask on a water bath (no naked flame!). Boil for 5 minutes with adsorbent charcoal and filter. The solution should be prepared freshly for each occasion since it does not keep well.

R 1l. *Iodine Solution.*

Dissolve 10 gm. potassium iodide in water. Add 10 gm. iodine crystals and shake to dissolve. Dilute with water to 1 litre.

R 1m. *Lime Water.*

Dissolve 1·5 gm. slaked lime (calcium hydroxide) at room temperature in 1 litre of water.

R 1n. *Millon's Solution.*

Dissolve 1 ml. mercury in 20 ml. concentrated nitric acid, warming slightly if necessary. Add twice the volume of distilled water, stand overnight and filter. Carry out the whole preparation in a fume cupboard.

An improved version (Cole's) of Millon's test is as follows:—

Dissolve 100 ml. pure sulphuric acid in 800 ml. distilled water. Carefully dissolve in this 100 gm. mercuric sulphate, filter, and make up to 1 litre. Boil suspected protein solution with an equal volume of this mercuric sulphate—sulphuric acid, and cool. Add 1–2 drops 1% sodium nitrite solution and warm gently. A red precipitate or coloration is given if protein is present.

R 1p. *Ninhydrin Solution.*

Dissolve 0·04 gm. commercial solid in 20 ml. distilled water.

R 1q. *Picric-picrate Reagent.*

For colorimetric estimation of glucose.

A. 36 gm. picric acid

Dissolve in 400 ml. hot water.

B. 16·5 gm. anhydrous sodium carbonate

Dissolve in 100 ml. water.

To 5 ml. of glucose solution, add 4 ml. of solution A and 1 ml. of solution B.

R 1r. *Schultz's Solution.*

A. 100 gm. zinc

Dissolve in 300 ml. pure hydrochloric acid. Evaporate down to 150 ml.

B. 12 gm. potassium iodide+0.15 gm. iodine crystals.

Dissolve in as little distilled water as possible.

Mix the two solutions.

R 1s. *Sealing Wax.*

A simple mixture for making air-tight joints.

35 gm. beeswax

50 gm. vaseline

Melt together, and into the mixture stir gradually 15 gm. powdered resin.

R 1t. *Soap Solution.*

A convenient medium for mounting moulds for microscopic examination. Dissolve a pure soap in water until it causes immediate wetting of cotton wool threads.

R 1u. *Stained Fibrin.*

Whip some fresh blood with a bundle of twigs or shake in a flask with glass beads until fibrin coagulates on them. Place the fibrin on a sieve and wash thoroughly in running water (or use commercial fibrin). Chop finely, place in carmine (1 gm. carmine+1 ml. ammonia solution+500 ml. water) or Congo Red for 24 hours and wash away excess stain.

R 1v. *Starch Paste.*

Mix 1 gm. potato starch into a paste with 10 ml. cold water,

and pour into 80 ml. boiling water, stirring well. Wash out beaker with another 10 ml. cold water and pour into boiling mixture. Continue boiling for 1 minute longer, then allow to cool.

R 2. Collection and Culture Methods.

R 2a. *Amoeba.*—Collect from mud, silt, etc. from ditches or ponds. This is not as easy as it sounds, and the inexperienced biologist will do better to buy from a dealer. Boil 10 wheat grains in 1 pint of water and cool. Add the amoebae, and every few weeks add a few extra grains. Every 3 or 4 months, set up a new culture, inoculating from the old.

R 2b. *Artemia salina* (brine shrimp).—Buy eggs from a dealer. The animals can easily be reared in very salty water.

R 2c. *Bacteria.*—The difficulty is not to get specimens, but to avoid getting too many from the air, fingers, dust, etc. Defined species can be obtained from Lister Institute. A suitable culture medium is nutrient agar. Dissolve 3 gm. peptone, 1 gm. beef extract, 1 gm. common salt, in 300 ml. water in a litre flask. Add 6 gm. agar-agar which has been soaked in water overnight. Heat in a steam oven until the agar is dissolved. Make slightly alkaline with sodium carbonate solution (purple with litmus). Filter through filter paper, keeping hot in a steam oven until filtration is completed (probably at least a day). Place the filtrate in a flask, plug its neck with cotton wool, heat in a steam oven for at least ½ hour on each of three successive days. Probably most teachers will prefer to buy sterile tubes of medium size from a dealer. All apparatus used in bacteriological experiments should be sterilised in a steam oven, dry oven, or flame; and great care should be taken that bacteria do not enter from the air (e.g. cotton wool plugs should be singed in a flame). *See also* C 4g; D 1c–g, k, l.

R 2d. *Butterflies and Moths.*—May be grown from eggs in cages. These are of various forms, and may be purchased from a dealer or improvised by making a box with wooden

base, glass front, and other sides of gauze. Feed with the plant food normal for the species.

R 2e. *Calliphora erythrocephola* (blowfly or bluebottle).—Can be bought as larvae ("gentles") from fishermen's shops. Keep in moist sawdust in a container covered with gauze. Larvae pupate and then develop into imagines.

R 2f. *Daphnia* (water flea).—Often present in pond water. Otherwise obtain from a dealer. To the water add at intervals a little thick paste of malted milk powder, rotten eggs, or filtered decoction of fowl dung (3 gm. boiled in 1 litre water, filtered and exposed for some days to sunlight). Keep several cultures growing if needed for feeding *Hydra,* etc.

R 2g. *Drosophila melanogaster* (vinegar fly).—Very useful for breeding experiments and easily reared. Obtain from a dealer or research laboratory. Sterilise ½ pint milk or similar bottles by placing in a steam oven for 1 hour. Place in the bottles a little of a mixture of equal quantities of cooked maize meal and treacle with the addition of some yeast, or part of an over-ripe banana. Make over the food a platform of corrugated paper on which the flies may rest. Plug the bottles with singed cotton wool and cover with rubber-banded grease-proof paper. A new generation is produced approximately each fortnight, and may be examined with low power microscope while anaesthetised with ether. The flies may be transferred from bottle to bottle by taking advantage of their positive phototaxis.

R 2h. *Gammarus chevreuxi* (brackish water shrimp).—Very useful for breeding experiments. Obtain from a dealer or research laboratory. Keep in small containers of brackish water (1/5th sea water, 4/5th tap water) with dead elm leaves for food. Change water when a scum forms. Place young animal on a slide by means of a pipette, and examine microscopically (using a low power objective).

R 2j. *Hydra.*—Can usually be obtained by careful examination of pond weeds of various sorts. Otherwise obtain from

a dealer. Green hydra (*H. viridis*) can live for a long time merely in a jar of pond water since its green alga-cells photosynthesise. Common hydra (*H. fusca*) does well in pond water, with a little *Elodea* and plenty of *Daphnia* added at intervals.

R 2k. *Lumbricus* (earthworm).—Usually found on the surface of lawns after rain, especially in the evening. May often be attracted to the surface by spraying ground with a dilute mustard solution. In winter keep the worms in a heap of moist soil and leaf mould, covered with old sacking.

R 2l. *Paramecium.*—Can usually be obtained by leaving hay in water for 3–6 weeks. It is not easy to get a pure culture, and many people prefer to purchase from a dealer. Culture in a medium of chopped hay simmered in water and cooled. Subculture about every 6 weeks. Do not confuse with *Colpidium* (which is smaller).

R 2m. *Planarians.*—May be collected by leaving a dead frog (split open) or a bottle containing boiled earthworm in a stream or pond. Next day examination will usually reveal many planarians near or on the bait. They may easily be kept in an aquarium, being fed by meat fragments, etc.

R 2n. *Trout.*—Eggs may be collected from streams, but it is easiest to obtain from a dealer. They require for their development running water. This can be arranged by placing a bung with two glass tubes into the mouth of a large container (e.g. Winchester), one tube reaching nearly to the bottom and the other only just dipping below the level of the water containing the eggs. The former tube is connected to the tap, thus bringing a slow constant stream of water into the flask; while the latter tube is bent over the sink, and water drips out from it at the same rate. The hatching may take anything from 50–100 days, according to the temperature. In about 6 weeks the yolk will all have been used, and for a little while the fish may be fed on sieved hard-boiled egg white. It is difficult to rear the fish for very long, and they should be liberated in streams after their development has been examined.

R 3. Preservation of Plant Specimens.

R 3a. Where possible preserve the whole plant, not merely the part above ground. Try to include shoot, root, flower and fruit.

R 3b. Ordinary herbs should be dried by pressing between layers of absorbent paper (it is worth buying proper drying paper). Compress by heavy books or between strapped boards or in a press of the type often used for lino-cut printing. The paper should be changed daily until drying is complete. If several plants are drying at once, separate by sheets of corrugated paper or cardboard.

R 3c. Mount dried specimens on standard herbarium paper, either sticking with gum tragacanth, or holding in place by strips of sticky paper.

R 3d. Keep moth balls in the storage cabinet, to discourage insect attacks.

R 3e. If specimens are to be preserved for some years, it is wise to spray them with a 5% alcoholic mercuric chloride solution, which prevents the growth of fungi, etc. (But do not do this if young children have access to the sheets, since the chloride is poisonous.)

R 3f. Colours of flowers can often be preserved by drying the specimens in a tray of sand, heated to 90° C. and allowed to cool with the specimen completely and carefully surrounded by the sand.

R 3g. Succulent herbs are best immersed in boiling water for a few moments before drying.

R 3h. Conifers tend to lose their leaves easily. This can often be prevented by immersing in 10% formalin solution for ½ hour before drying.

R 3j. Water herbs and algae are best mounted by placing in a shallow tray of water, slipping a sheet of paper under the

specimen, and floating the specimen on to it. By removing the paper carefully and draining, neat preparations may be obtained. Cover with butter muslin and press as for land plants; removing, drying and replacing the muslin as often as may be necessary. This prevents algae from adhering to the drying paper. For uniformity, paste the paper with specimen on an herbarium sheet.

R 4. Aquarium Management.

R 4a. Use fairly flat aquaria, not very deep ones—a high surface/volume ratio helps aeration.

R 4b. If old accumulator glasses are used, scour repeatedly until clean, before use—many aquatic animals are easily killed by traces of acid or other impurities.

R 4c. Place in diffused light—direct sunlight encourages the growth of small green algae on the sides of the aquarium.

R 4d. If possible, let light fall onto the water from above, with at most one side of the aquarium transparent—this approximates to natural conditions.

R 4e. Cover the bottom of the aquarium with gravel and washed sand—no natural pond ever had a glass bottom!

R 4f. Build little arches and caves with stones—many animals like an occasional retreat.

R 4g. Introduce water into the aquarium carefully, otherwise the sand and gravel will be unduly disturbed.

R 4h. Place aquarium plants in little pots or twine around glass rods—this facilitates changing.

R 4j. Let the plants become well established before introducing animals—a new house is always more cosy if it is furnished!

R 4k. Do not mix predaceous animals (e.g. *Dytiscus*, dragonfly larvae, etc.) with others—otherwise there will be heavy casualties.

R 4l. Maintain a correct balance of animals and plants—the plants help to aerate the water, and the animals produce carbon dioxide which is of value to the plants.

R 4m. If artificial aeration is used, try to get an electric type —these are fairly foolproof, and most laboratories harbour a few fools!

R 4n. Introduce air into the aquarium *via* a porous diffuser —this avoids excessive disturbance.

R 4p. Fear not to experiment with marine and tropical aquaria—you learn a lot even in failing, and it is good fun!

R 5. Use of Microscope.

R 5a. Become thoroughly familiar with the microscope's structure and the function, care and adjustment of its components.

R 5b. If a substage condenser is affixed always use the plane side of the mirror. If there is no substage condenser, use the concave side of the mirror.

R 5c. Work with the iris diaphragm open as little as possible, since excessive light impairs definition and hinders observation.

R 5d. Examine the object first with the low power objective, using the high power only if and when necessary.

R 5e. Unless you are sure that the objectives are complementary, raise them about half an inch before swinging the nose-piece round from low to high power to make sure that objective does not touch the slide by error.

R 5f. To focus with the high power objective, bring it down very close to the object, and rack *upwards* away from slide.

R 5g. Take care that none of the mounting liquid finds its way to the top of the cover slip, since it might get on to the high power objective, and will in any event blur vision.

R 5h. When using the microscope keep *both* eyes open. This requires a little practice, but causes less eye strain and facilitates the drawing of objects.

R 6. Plant Sectioning.

R 6a. The best type of razor is one specially made for the purpose, hollow ground on only one surface. But a "cut throat" is perfectly suitable, and often gives better results than a cheap botanical razor, being made of finer steel and thus taking a sharper edge.

R 6b. The most important thing is to have the razor *really* sharp, and to keep it sharp by constant honing and stropping.

R 6c. For honing, wet the stone with glycerin or oil (according to the type of stone) and lay the razor with its flat surface on it at an angle of 45° with the length of the stone. Pull the razor edge first towards you with the right hand, using the fingers of the left hand to press the blade gently on the stone. Now turn the razor over to the other surface, taking great care to use the *back* of the blade as fulcrum. Now similarly push the razor edge first away from you. Repeat as often as necessary.

R 6d. For stropping, move the razor back-first up and down the strop, keeping the strop really taut, and taking great care to use the *back* of the blade as fulcrum.

R 6e. Trim the material first with a scalpel or old razor, *not* with the sectioning razor.

R 6f. Practice cutting sections on a piece of potato tuber.

R 6g. Always keep the material and razor thoroughly wet with water or alcohol, to avoid the formation of air bubbles.

R 6h. Hold the material perfectly vertical in the left hand, and cut sections towards you, with a diagonal sliding motion.

R 6j. When several sections are on the razor, slide them off

with the finger tip or a camel hair brush into a watchglass of water or alcohol (or other medium).

R 6k. Select the thinnest sections for examination, rather than the most complete ones.

R 6l. If the material is thin or soft, place it in a slit in elder pith, or between two pieces of pith, and hold firmly while cutting pith and section together.

R 6m. Material may be hardened by soaking in concentrated alcohol. In this case, keep the material and razor wet with alcohol while sectioning.

R 6n. Transfer the section from watchglass to slide with a camel hair brush, add as little as possible of the mounting medium (usually dilute glycerin), and let down the coverslip on to it very gently.

R 7. Illustrative Models.

R 7a. *Diaphragm Breathing.*—The action of the diaphragm in breathing may be illustrated by means of a simple apparatus. The thorax is represented by a bell jar; the wind pipe by glass tubing passing into the "thorax" through a bung, and terminating in a ⅄-piece, of which the lower arms represent the bronchi; the lungs by toy rubber balloons attached to the "bronchi"; and the diaphragm by a rubber sheet covering the base of the bell jar. Movement of the "diaphragm" down and up causes "breathing in" and "breathing out". An improved version is made by using a bell jar only slightly larger than the expanded balloons, and filling the space between with water, representing the lymph around the lungs.

R 7b. *Rib Breathing.*—Take an aquarium with an outlet at one end, and stand it upright so that the outlet is on the top. Pass glass tubing through the outlet, and attach rubber balloons to represent lungs, as in R 7a. Cover the front of the aquarium with a glass sheet, and hinge along the top edge by means of adhesive tape. Cut out "American cloth" of a suit-

able shape to go loosely round the other three edges, and attach by adhesive tape to the aquarium rim and the glass sheet. By movement of the glass plate about its hinge, raising and lowering of ribs may be simulated, with consequent filling and emptying of the "lungs".

R 7c. *Stoma.*—A model showing action of guard cells may be made from a bicycle inner tube. While it is part-pumped, fold it in two and bind each end lightly. Now pump more strongly. This is comparable to the absorption of more water by the guard cells by osmosis. The gap in the centre of the tube (i.e. the "stoma") opens more widely.

R 7d. *Blood Circulation.*—A winchester of water represents the heart, the "blood pressure" being raised by raising the winchester. Water siphons along a glass and rubber tube, off which rise two vertical glass tubes, representing arteries and veins respectively, and separated by a rubber tube which may be clipped to represent capillary resistance. Water rises higher in the first tube than the second (i.e. "arterial pressure" greater than "venous pressure").

R 7e. *Skeletal Movement.*—Models illustrating skeletal movement can be made quite easily. The bones are represented by plywood, the muscles by rubber elastic or curtain springs and the tendons by cord. Joints are pivoted by pins. Suitable systems for models are those of the arm, foot, and neck and head.

R 7f. *Ear.*—Cut out of plywood representations of the ossicles and other ear parts. Attach the fixed parts to a baseboard, and pivot the "ossicles" with pins. The tympanum and other membranes are represented by slightly stretched elastic pinned to the edge of the plywood parts. Movement of the tympanum is transmitted by the "ossicles" to the "inner membrane".

R 7g. *Eye.*—With carborundum and water grind a panel about 4″ square on a gold-fish bowl to provide a translucent screen representing the "retina". Opposite this, mark out a

circle (3" diameter) as "cornea", and black enamel the remainder of the interior. As "iris", use a circular disc of metal with a central hole as "pupil". This can be attached by spring clips to the rim of the bowl. A glass lens can be held behind the iris by metal clips. Fill the bowl with water to represent the transparent eye contents. A lighted candle in front of the bowl will cast an image on the screen.

R 7h. *Statocyst.*—In a hollow india-rubber ball (the "statocyst") make cuts so that a small solid brass ball (the "statolith") may be introduced. By means of the flap also place a pair of wide headed brass-screws ("sensitive cells") through the rubber ball in each of three or four different areas. The heads of the screws should be inside the ball, and sufficiently close for the brass ball to touch both members of a pair at once. Connect up each pair of screws by their projecting points to an electric cell and an electric bell. As the india-rubber ball ("statocyst") is twisted and turned, the brass ball ("statolith") completes the circuits of different pairs of screws ("sensitive cells") thus causing different bells to ring (i.e. "nervous reactions").

R 7j. *Cellular Structure.*—Spread on a clean glass plate a 10% solution of gelatine, and allow to set. Now from a pipette, place on the gelatine drops of 8% potassium ferrocyanide solution in a grid formation, neighbouring drops being separated by 5 mm. When the gelatine dries out, the ferrocyanide is seen to have diffused in such a way as to simulate polygonal cell formation. The "cell-walls" are often seen more clearly if an image of the plate is projected onto a screen.

Similarly cell-formation may be simulated by sowing drops of salt solution coloured with indian ink, upon a plate covered by a thin layer of water.

R 7k. *Periodic Structure.*—To 5 ml. of 10% gelatine solution add 1 drop of concentrated calcium nitrate solution. Pour evenly over a glass plate and allow to set. As soon as set, place on the plate either a drop or a thin line of a concentrated tri-

sodium phosphate solution. A periodic precipitate of calcium phosphate (Liesegang's phenomenon) is obtained as a result of the diffusion of the phosphate. Often the lines of precipitation are very close together, so that the precipitate acts as a grating and produces spectra. This type of periodicity is responsible for a good deal of the coloration and iridescence of insects, feathers, pearls, etc.

R 71. *Nuclear Division.*—Cover a glass plate with a semi-saturated solution of potassium nitrate ("cytoplasm"). Place in the centre a drop of the same solution coloured with indian ink ("nucleus") and on each side of this a more faintly coloured drop of a more concentrated solution ("poles"). The indian ink particles may move in such a way as to simulate nuclear division. A good deal of practice may be necessary before success is achieved.

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